PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 16 June 2000 (16.06.00)	
International application No. PCT/FI99/00870	**Applicant's or agent's file reference** 31531
International filing date (day/month/year) 20 October 1999 (20.10.99)	**Priority date** (day/month/year) 23 October 1998 (23.10.98)
Applicant YLIHONKO, Kristiina et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

08 May 2000 (08.05.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was
[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland** Facsimile No.: (41-22) 740.14.35	Authorized officer Manu Berrod Telephone No.: (41-22) 338.83.38

Applicant's or agent's file reference	31531	International application No. PCT/FI99/00870

INDICATIONS RELATING TO DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 18, lines 17-21

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet ☐

Name of depositary institution

Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ)

Address of depositary institution *(including postal code and country)*

Mascheroder Weg 1b, D-38124 Braunschweig, Germany

Date of deposit	Accession Number
14 October 1998	DSM 12451, DSM 12452

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet ☐

In respect of those designations in which a European patent is sought, a sample of the deposited biological material will be made available as provided in Rule 28(3) EPC, until the publication of the mention of the grant of the European patent or for 20 years from the date of filing if the application is refused or withdrawn or deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
☒ This sheet was received with the international application	☐ This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

PATENTTI- JA REKISTE[redacted]ALLITUS
Patentti- ja innovaatiolinja

T[redacted]KIMUSRAPORTTI
Search Report

HAKEMUSNUMERO	LUOKITUS
982295	C 12 N 15/31, 15/52, 15/76, C 07 H 15/252, C 12 P 19/56, C 12 N 1/21
	// (C 12 N 15/31, C 12 R 1:465) □ jatkuu kääntöpuolella

TUTKITTU AINEISTO

Patenttivirastojen julkaisut
FI, SE, NO, DK, DE, CH, EP, WO, GB, US:

FI, SE, NO, DK, julkaisut viraston julkaisukokoelmasta luokista C 12 N 15/31, 15/52, 15/76, C 07 H 15/252, C 12 P 19/56

Tietokannoista World Patent Index, EPO Documentation, Patent Abstracts of Japan, US full text (Epoque)

□ jatkuu kääntöpuolella

Muu aineisto

Tietokannoista Caplus, Biosis, Medline, Registry (STN)

□ jatkuu kääntöpuolella

VIITEJULKAISUT

Kategoria*)	Julkaisun tunnistetiedot	Koskee vaatimuksia
A	Ylihonko, K. et al., WO 96/10581, C 07 K 14/36	1-15
A	Ylihonko, K. et al., Microbiology 142 (1996) 1965 - 1972	1-15
A	Ylihonko, K. et al., Mol. Gen. Genet. 251 (1996) 113 - 120	1-15
A	Torkkell, S. et al., Mol. Gen. Genet. 256 (1997) 203 - 209	1-15

□ jatkuu kääntöpuolella

*) X Patentoitavuuden kannalta merkittävä julkaisu yksinään tarkasteltuna
Y Patentoitavuuden kannalta merkittävä julkaisu, kun otetaan huomioon tämä ja yksi tai useampi samaan kategoriaan kuuluva julkaisu
A Yleistä tekniikan tasoa edustava julkaisu, ei kuitenkaan patentoitavuuden este

Päiväys	Tutkija
5.7.1999	Stiina Kaikkonen

PATENT COOPERATION TREAT

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 31531	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/FI99/00870	International filing date *(day/month/year)* 20.10.1999	Priority date *(day/month/year)* 23.10.1998
International Patent Classification (IPC) or national classification and IPC7 C 07 K 14/36, C 12 N 15/31, C 12 P 19/56		
Applicant Galilaeus Oy et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 3 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of ______ sheets.

3. This report contains indications relating to the following items:

   I ☒ Basis of the report

   II ☐ Priority

   III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

   IV ☐ Lack of unity of invention

   V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

   VI ☐ Certain documents cited

   VII ☐ Certain defects in the international application

   VIII ☐ Certain observations on the international application

Date of submission of the demand 08.05.2000	Date of completion of this report 12.01.2001
Name and mailing address of the IPEA/SE Patent- och registreringsverket Box 5055 S-102 42 STOCKHOLM Telex 17978 PATOREG-S Facsimile No. 08-667 72 88	Authorized officer Patrick Andersson/EÖ Telephone No. 08-782 25 00

Form PCT/IPEA/409 (cover sheet) (January 1998)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/FI99/00870

I. Basis of the report

1. With regard to the **elements** of the international application:*

[X] the international application as originally filed

[ ] the description:
pages ______________________________, as originally filed
pages ______________________________, filed with the demand
pages ______________________________, filed with the letter of ______________

[ ] the claims:
pages ______________________________, as originally filed
pages ______________________________, as amended (together with any statement) under article 19
pages ______________________________, filed with the demand
pages ______________________________, filed with the letter of ______________

[ ] the drawings:
pages ______________________________, as originally filed
pages ______________________________, filed with the demand
pages ______________________________, filed with the letter of ______________

[ ] the sequence listing part of the description:
pages ______________________________, as originally filed
pages ______________________________, filed with the demand
pages ______________________________, filed with the letter of ______________

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language ______________ which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).
[ ] the language of publication of the international application (under Rule 48.3(b)).
[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/ or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

[ ] contained in the international application in written form.
[ ] filed together with the international application in computer readable form.
[ ] furnished subsequently to this Authority in written form.
[ ] furnished subsequently to this Authority in computer readable form.
[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
[ ] The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. [ ] The amendments have resulted in the cancellation of:

[ ] the description, pages ______________
[ ] the claims, Nos. ______________
[ ] the drawings, sheet/fig ______________

5. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2 (c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item I and annexed to this report.*

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-15	YES
	Claims		NO
Inventive step (IS)	Claims	1-15	YES
	Claims		NO
Industrial applicability (IA)	Claims	1-15	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

The claimed invention relates to a gene cluster for the anthracycline biosynthetic pathway of Streptomyces nogalater comprising one 10kb fragment and one 7kb flanked BglII fragment of S. nogalater genome. The cluster, or parts thereof, can be used for synthesis of hybrid antibiotics.

The following document is considered relevant:
D1) Torkell S et al., "Characterization of Streptomyces nogalater genes encoding enzymes involved in the glycosylation steps in nogalamycin biosynthesis", Mol Gen Genet, 1997, vol 256, pages 203-209.

D1 discloses the characterization of S. nogalater genes involved in the glycosylation steps in nogalamycin biosynthesis. The genes are studied by inserting parts of a gene cluster into a strain of S. galilaeus (H039), thereby producing hybrid compounds. A sequence similarity search with the sequence for the presently claimed snoal gene indicates that snoaL is known as a sub-sequence of sequence AF187532 related to D1.

In relation to the subject matter of the other claims D1 is considered to represent the general state of the art and has no particular relevance. Thus, the invention according to claims 1-15 is novel, inventive and industrially applicable.

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING SUBMISSION OR TRANSMITTAL OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

To:

OY JALO ANT-WUORINEN AB
Iso Roobertinkatu 4-6 A
FIN-00120 Helsinki
FINLANDE

Date of mailing (day/month/year) 25 November 1999 (25.11.99)	
Applicant's or agent's file reference 31531	**IMPORTANT NOTIFICATION**
International application No. PCT/FI99/00870	International filing date (day/month/year) 20 October 1999 (20.10.99)
International publication date (day/month/year) Not yet published	Priority date (day/month/year) 23 October 1998 (23.10.98)
Applicant GALILAEUS OY et al	

1. The applicant is hereby notified of the date of receipt (except where the letters "NR" appear in the right-hand column) by the International Bureau of the priority document(s) relating to the earlier application(s) indicated below. Unless otherwise indicated by an asterisk appearing next to a date of receipt, or by the letters "NR", in the right-hand column, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).
2. This updates and replaces any previously issued notification concerning submission or transmittal of priority documents.
3. An **asterisk(*)** appearing next to a date of receipt, in the right-hand column, denotes a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b). In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.
4. The **letters "NR"** appearing in the right-hand column denote a priority document which was not received by the International Bureau or which the applicant did not request the receiving Office to prepare and transmit to the International Bureau, as provided by Rule 17.1(a) or (b), respectively. In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

Priority date	Priority application No.	Country or regional Office or PCT receiving Office	Date of receipt of priority document
23 Octo 1998 (23.10.98)	982295	FI	16 Nove 1999 (16.11.99)

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland** Facsimile No. (41-22) 740.14.35	Authorized officer Catherine Massetti Telephone No. (41-22) 338.83.38

PA●ENT COOPERATION TREA

12.05.2000

From the INTERNATIONAL BUREAU

PCT

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

To:
OY JALO ANT-WUORINEN AB
Iso Roobertinkatu 4-6 A
FIN-00120 Helsinki
FINLANDE

Date of mailing (day/month/year) 04 May 2000 (04.05.00)		
Applicant's or agent's file reference 31531	**IMPORTANT NOTICE**	
International application No. PCT/FI99/00870	**International filing date** (day/month/year) 20 October 1999 (20.10.99)	**Priority date** (day/month/year) 23 October 1998 (23.10.98)
Applicant GALILAEUS OY et al		

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
   JP,US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
   EP

   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
   04 May 2000 (04.05.00) under No. WO 00/24775

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, **a demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the **national phase**, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer J. Zahra
Facsimile No. (41-22) 740.14.35	Telephone No. (41-22) 338.83.38

Continuation of Form PCT/IB/308

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

Date of mailing (day/month/year) 04 May 2000 (04.05.00)	IMPORTANT NOTICE
Applicant's or agent's file reference 31531	International application No. PCT/FI99/00870

The applicant is hereby notified that, at the time of establishment of this Notice, the time limit under Rule 46.1 for making amendments under Article 19 has not yet expired and the International Bureau had received neither such amendments nor a declaration that the applicant does not wish to make amendments.

PCT/FI99/00870

PATENT COOPERATION TREATY

04.07.2000

From the INTERNATIONAL BUREAU

PCT

INFORMATION CONCERNING ELECTED OFFICES NOTIFIED OF THEIR ELECTION

(PCT Rule 61.3)

To:

OY JALO ANT-WUORINEN AB
Iso Roobertinkatu 4-6 A
FIN-00120 Helsinki
FINLANDE

Date of mailing (day/month/year) 16 June 2000 (16.06.00)		
Applicant's or agent's file reference 31531	**IMPORTANT INFORMATION**	
International application No. PCT/FI99/00870	**International filing date** (day/month/year) 20 October 1999 (20.10.99)	**Priority date** (day/month/year) 23 October 1998 (23.10.98)
Applicant GALILAEUS OY et al		

1. The applicant is hereby informed that the International Bureau has, according to Article 31(7), notified each of the following Offices of its election:

   EP :AT,BE,CH,CY,DE,DK,ES,FI,FR,GB,GR,IE,IT,LU,MC,NL,PT,SE
   National :JP,US

2. The following Offices have waived the requirement for the notification of their election; the notification will be sent to them by the International Bureau only upon their request:

   None

3. The applicant is reminded that he must enter the "national phase" **before the expiration of 30 months from the priority date** before each of the Offices listed above. This must be done by paying the national fee(s) and furnishing, if prescribed, a translation of the international application (Article 39(1)(a)), as well as, where applicable, by furnishing a translation of any annexes of the international preliminary examination report (Article 36(3)(b) and Rule 74.1).

   Some offices have fixed time limits expiring later than the above-mentioned time limit. For detailed information about the applicable time limits and the acts to be performed upon entry into the national phase before a particular Office, see Volume II of the PCT Applicant's Guide.

   The entry into the European regional phase is postponed **until 31 months from the priority date** for all States designated for the purposes of obtaining a European patent.

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland** Facsimile No. (41-22) 740.14.35	Authorized officer: Manu Berrod Telephone No. (41-22) 338.83.38

CORRECTED VERSION*

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : C07K 14/36, C12N 15/31, C12P 19/56	A1	(11) International Publication Number: **WO 00/24775** (43) International Publication Date: 4 May 2000 (04.05.00)

(21) International Application Number: PCT/FI99/00870

(22) International Filing Date: 20 October 1999 (20.10.99)

(30) Priority Data:
982295 23 October 1998 (23.10.98) FI

(71) Applicant *(for all designated States except US)*: GALILAEUS OY [FI/FI]; Kairiskulmantie 10, FIN-20760 Piispanristi (FI).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: YLIHONKO, Kristiina [FI/FI]; Betonimiehenkatu 13, FIN-20780 Kaarina (FI). TORKKELL, Sirke [FI/FI]; Välppätie 3 B 39, FIN-20540 Turku (FI). PALMU, Kaisa [FI/FI]; Eerikinkatu 41 B 42, FIN-20100 Turku (FI). HAKALA, Juha [FI/FI]; Elinantie 2 A 9, FIN-20540 Turku (FI).

(74) Agent: OY JALO ANT-WUORINEN AB; Iso Roobertinkatu 4-6 A, FIN-00120 Helsinki (FI).

(81) Designated States: JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.
With an indication in relation to deposited biological material furnished under Rule 13bis separately from the description.

(54) Title: GENE CLUSTER INVOLVED IN NOGALAMYCIN BIOSYNTHESIS, AND ITS USE IN PRODUCTION OF HYBRID ANTIBIOTICS

(57) Abstract

The present invention relates to the gene cluster for nogalamycin biosynthesis derived from *Streptomyces nogalater*, and the use of the genes therein to obtain novel hybrid antibiotics for drug screening.

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification [7] :
C07K 14/36, C12N 15/31, C12P 19/56

A1

(11) International Publication Number: WO 00/24775

(43) International Publication Date: 4 May 2000 (04.05.00)

(21) International Application Number: PCT/FI99/00870

(22) International Filing Date: 20 October 1999 (20.10.99)

(30) Priority Data:
982295 23 October 1998 (23.10.98) FI

(71) Applicant *(for all designated States except US)*: GALILAEUS OY [FI/FI]; Kairiskulmantie 10, FIN-20760 Piispanristi (FI).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: YLIHONKO, Kristiina [FI/FI]; Betonimiehenkatu 13, FIN-20780 Kaarina (FI). TORKKELL, Sirke [FI/FI]; Välppätie 3 B 39, FIN-20540 Turku (FI). PALMU, Kaisa [FI/FI]; Eerikinkatu 41 B 42, FIN-20100 Turku (FI). HAKALA, Juha [FI/FI]; Elinantie 2 A 9, FIN-20540 Turku (FI).

(74) Agent: OY JALO ANT-WUORINEN AB; Iso Roobertinkatu 4-6 A, FIN-00120 Helsinki (FI).

(81) Designated States: JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: GENE CLUSTER INVOLVED IN NOGALAMYCIN BIOSYNTHESIS, AND ITS USE IN PRODUCTION OF HYBRID ANTIBIOTICS

(57) Abstract

The present invention relates to the gene cluster for nogalamycin biosynthesis derived from *Streptomyces nogalater*, and the use of the genes therein to obtain novel hybrid antibiotics for drug screening.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

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Gene cluster involved in nogalamycin biosynthesis, and its use in production of hybrid antibiotics

Field of the invention

This invention relates to the gene cluster for nogalamycin biosynthesis derived from *Streptomyces nogalater*, and the use of the genes therein to obtain novel hybrid antibiotics for drug screening.

Background of the invention

Anthracyclines are antitumor antibiotics, mainly produced by *Streptomyces* sp. Daunomycin family of anthracyclines is commercially most important, since almost all of the around ten anthracyclines currently in clinical use, or in late clinical trials for cytotoxic drugs, belong to this family. Despite the long history of anthracyclines, three decades or so, the studies on their biosynthesis are still going on, and there is further interest to obtain novel molecules for the development of cancer chemotherapeutics. A method currently used for finding novel molecules for drug screening is genetic engineering. Cloning the genes for anthracycline biosynthesis facilitates the production of hybrid anthracyclines, as well as their use in combinatorial biosynthesis to generate novel molecules.

Nogalamycin, which was first described by Bhuyan and Dietz in 1965, is an anthracycline antibiotic produced by *Streptomyces nogalater*. It is highly active against tumor cells, whereas toxic properties of this compound have prevented its progress to clinical trials (Bhuyan and Smith, 1975). However, menogaril (7–O–methylnogarol) is a semisynthetic derivative of nogalamycin, and its value in the treatment of cancer has been studied (e.g. Yoshida *et al.*, 1996), the interest being now mainly in Japan. Structurally nogalamycin (Fig. 1) differs from most other anthracyclines, as e.g. from the daunomycin family, in two noteworthy features: (i) The stereochemistry at position nine is opposite, and (ii) it has a sugar moiety, in which nogalamine is attached at position 1 by a typical glycosidic bond, but it is also attached to carbon 2 by an

extraordinary C–C bond. Structural elucidation of nogalamycin was reported by Wiley *et al.* (1977). Furthermore, biosynthetic studies of nogalamycin have been published by Wiley *et al.* in 1978 giving information of the building blocks: The aglycone moiety is built from ten acetates; the neutral sugar, nogalose, is derived from glucose; and methyl groups of both of the sugars, nogalamine and nogalose, are transferred from methionine. The origin of nogalamine was not clearly solved by Wiley, but most probably nogalamine is also derived from glucose.

Molecular cloning of biosynthesis genes for anthracyclines has facilitated the studies on molecular genetics, providing tools for rational modifications of the structures, while also for surprising combinations with other antibiotics. Most of the interest has focused on daunomycin biosynthesis genes, as reported in several publications (Lomovskaya *et al.*, 1998; Rajgarhia and Strohl, 1997 and references therein). Some genes for aclacinomycin biosynthesis from *S. galilaeus* (Fujii and Ebizuka, 1997) and for rhodomycin biosynthesis from *S. purpurascens* (Niemi *et al.*, 1994) have been cloned as well. We have cloned the biosynthesis genes for nogalamycin, and successfully used the genes for producing hybrid anthracyclines. Most of the genes are involved in polyketide pathway, being responsible for the formation of a tricyclic intermediate, and they are reported in Ylihonko *et al.*, 1996a and b, and by Torkkell *et al.*, 1997. Despite the advances in molecular cloning, the biosynthetic pathway from glucose to sugars found in anthracyclines is still mainly hypothetical.

Regarding the genes for deoxyhexose pathway, Madduri *et al.* (1998) have reported that a gene derived from avermectin biosynthesis cluster caused the production of hybrid anthracyclines altering the sugar moiety when transferred into an *S. peucetius* mutant. The product obtained was epirubicin, a commercially important anthracycline. In this case a hydroxy group in the daunosamine moiety was in the opposite stereochemistry due to the action of an avermectin biosynthesis gene. *S. galilaeus* has been used as the host to prepare hybrid anthracyclines using the genes derived from rhodomycin pathway from *S. purpurascens* (Niemi *et al.*, 1994), and from nogalamycin biosynthesis cluster from *S. nogalater* (Ylihonko *et al.*, 1996a). The genes for nogalamycin pathway were used to generate the hybrid anthracycline production in *S. steffisburgensis* producing

typically steffimycin (Kunnari *et al.*, 1997). Previously, biosynthesis genes for actinorhodin have been expressed in *S. galilaeus* resulting in the formation of aloesaponarin (Strohl *et al.*, 1991). These hybrid compounds were modified in the aglycone moiety.

Summary of the invention

The present invention concerns a gene cluster of *Streptomyces nogalater*, most of the genes of the cluster being derived from the deoxyhexose pathway for nogalamine and nogalose. Expressing a DNA fragment of the said region in *S. galilaeus*, which produces aclacinomycins, hybrid anthracyclines are obtained, wherein the aglycone moiety is derived from *S. galilaeus*, whereas the sugar moiety is characteristic neither to *S. nogalater* nor to *S. galilaeus*. Furthermore, when inserting the gene included in said cluster, encoding a cyclase for nogalamycin, into a suitable plasmid construction, nogalamycinone is obtained, which is the aglycone of nogalamycin. Since the stereochemistry of nogalamycin differs from most other anthracyclines, using this gene enables the preparation of C–9 stereoisomers of the anthracycline molecules.

Detailed description of the invention

The experimental procedures of the present invention are methods conventional in the art. The techniques not described in detail here are given in the manuals by Hopwood *et al.* "Genetic manipulation of Streptomyces: a laboratory manual" The John Innes Foundation, Norwich (1985) and by Sambrook *et al.* (1989) "Molecular cloning: a laboratory manual". The publications, patents and patent applications cited herein are given in the reference list in their entirety.

The present invention concerns particularly the gene cluster for nogalamycin biosynthesis (*Sno*5–cluster) causing the production of hybrid antibiotics with modifications in the sugar moiety. The invention concerns in specific the use of the genes for nogalamine/nogalose biosynthesis to generate hybrid antibiotics modified in sugar moieties. The invention also concerns the use of a specific cyclase gene included in the

gene cluster of the invention, to generate the C–9 stereoisomers of typical anthracyclines.

The gene cluster according to the present invention is linked to the earlier reported clusters for nogalamycin biosynthesis. The starting point of the present invention was the gene cluster for nogalamycin chromophor (International Patent Application WO 96/10581). Subsequently, we have found some genes for the deoxyhexose pathway of nogalamycin biosynthesis (Torkkell *et al.*, 1997), and a part of the fragment comprising said genes was used to clone the genes for this invention.

The biosynthesis genes for nogalamycin can be isolated from *Streptomyces* sp., particularly from *S. nogalater*, which produces nogalamycin. Species which produce nogalamycin–like anthracyclines can also be used, e.g. *S. violaceochromogenes* producing arugomycin (Kawai *et al.*, 1987), or *S. avidinii* producing avidinorubicin (Aoki *et al.*, 1991).

Genomic DNA of a *Streptomyces* strain carrying the genes for nogalamycin biosynthesis is used in preparing a genomic library. Suitable gene fragments for cloning may be obtained by any frequently digesting restriction enzyme. Typically *Sau*3AI is used. The isolated fragments could be inserted by ligation in any *Escherichia coli* vector such as a plasmid, a phagemid, a phage, or a cosmid. A cosmid vector is preferred since it enables the cloning of large DNA fragments. A cosmid vector such as pFD666 (ATCC No. 77286) is suitable for this purpose, as it enables cloning of the fragments of about 40 kb. The *Bam*HI site of pFD666, giving sticky ends to the *Sau*3AI fragments may be used for cloning. Commercially available kits may be used to pack the DNA in phage particles. Various *E. coli* strains can be used for the infection by the DNA packed. An appropriate *E. coli* strain is, e.g. XL1Blue MRF', which is deficient in several restriction systems.

Using *E. coli* as a host strain for the genomic library, hybridization is an advantageous screening strategy. The probe for hybridization may be any known fragment derived from the nogalamycin gene cluster, but a short fragment of about 1 kb derived from one

end of the biosynthetic region previously cloned is preferred. Colonies for the genomic library are transferred for filter hybridization to membranes, preferably to nylon membranes. Since the average size for a genomic DNA fragment is 40 kb, 2300 colonies gave 99.99% probability to find the expanded region for nogalamycin biosynthesis. Any method for hybridization may be used but, in particular, the DIG System (Boehringer Mannheim, GmbH, Germany) is useful. Since the probe is homologous to the hybridized DNA, it is preferable to carry out the stringent washes of hybridization at 70°C in a low salt concentration according to Boehringer Mannheim's manual "DIG System User's Guide for Filter Hybridization". At least 80% homology is suggested to be needed for a DNA fragment to bind a probe in the conditions used for washes.

Using this protocol, seven clones out of about 5000 gave positive signals, and were picked up for DNA isolation. Restriction mapping is an appropriate technique for characterizing the clones. The positive clones may be digested with convenient restriction enzymes to demonstrate the physical linkage map of the DNA fragments. The cosmid used for cloning was a shuttle cosmid replicating in both *E. coli* and *Streptomyces* sp. However, the transfer of the recombinant cosmids in *S. lividans* TK24, which is a typically used laboratory strain in cloning *Streptomyces*, resulted in deletions, and was omitted. Instead, we rather used in the expression studies the plasmid pIJ486, a high copy number *Streptomyces* plasmid. However, any plasmid being able to stably replicate in *Streptomyces* may be used for this purpose.

Two *Bgl*II fragments of one of the clones were separately inserted into pIJ486 vectors, and the two plasmids obtained were transferred into a primary host, *S. lividans* TK24. The recombinant plasmids obtained (pSY42 and pSY43), containing a 10 kb and a 7kb fragment from *S. nogalater* genomic DNA, respectively, were isolated from the primary host and further introduced into other *Streptomyces* strains by protoplast transformation. The recombinant plasmid containing the 10 kb fragment caused the production of hybrid anthracyclines in the *S. galilaeus* mutant strain H039, which endogenously produces aklavinone-rhodinose-rhodinose-rhodinose. A few other *S. galilaeus* strains (H075, H026, H063) mutated in deoxyhexose pathway for sugars in aclacinomycin were used in

transformation, and new hybrid compounds were obtained. Since the structure of nogalamycin is almost unique among anthracyclines, the plasmids could be transferred to other anthracycline-producing strains, such as *S. peucetius*, which produces daunomycin, and *S. purpurascens*, which produces rhodomycins, to modify the structures of the characteristic antibiotics.

As the cloned cluster was linked to nogalamycin biosynthesis region already known, its ability to generate the modification in sugar moiety suggested the presence of the genes for deoxyhexose pathway. However, sequencing is necessary to deduce the function of the genes in the cluster cloned. The DNA fragments of 10 kb and 7 kb were further inserted into the plasmid pSL1190 for subcloning. Sequencing strategies such as a deletion set of the DNA fragments, shotgun cloning or primer walking could be used, but we prefer to use restriction fragments for subcloning. Using ABI PRISM system (Perkin–Elmer) for sequencing it is possible to get 500 to 700 bases per one reaction, which means that about 1 kb fragments sharing overlapping bases are needed for sequencing. For this purpose, 27 subclones were constructed.

Sequencing of the flanked *Bgl*II fragments consisting of about 16000 bp revealed 15 complete ORFs. The sequence analysis can be made by any computer based program, such as GCG (Madison, Wisconsin, USA) package. According to the present invention the putative gene functions as deduced from the sequence homology of those available in the libraries are

aminotransferase (*sno*gI), not completed

1. dTDP–glucose synthase (*sno*gJ)
2. aminomethyl transferase (*sno*gA)
3. polyketide cyclase, (*sno*aM)
4. a gene of deoxyhexose pathway, unknown (*sno*gN)
5. hydroxylase, (*sno*aG)
6. dTDP–4–dehydrorhamnose reductase (*sno*gC)
7. dTDP–glucose 4,6–dehydratase (*sno*gK)
8. NAME cyclase (*sno*aL)
9. unknown (*sno*K)

10. glycosyl transferase, GTF (*sno*gD)
11. unknown (*sno*W)
12. glycosyl transferase, GTF (*sno*gE)
13. unknown (*sno*L)
14. unknown (*sno*O)
15. C–7 ketoreductase (*sno*aF)
    unknown (*sno*N), not completed

Gene designations: **g** means that the gene involved in biosynthesis of the glycosidic proportion including glycosyl transferases, whereas a points out that the gene is needed for the formation of the aglycone moiety.

Considering the proposed biosynthetic pathway for nogalamycin shown in Fig 3. we are able to cause several changes for the structures of antibiotics by the genes identified, including *sno*aL, responsible for the cyclization of the fourth ring of the aglycone moiety while determining the stereochemistry of the anthracyclinone, and the genes affecting the formation of nogalamine and nogalose (*sno*gJ, *sno*gK, *sno*gN, *sno*gC, *sno*gA), and, in addition, the genes responsible for joining the sugar residues to the aglycone moiety (*sno*gD and *sno*gE).

These genes could be separately inserted in a vector using suitable restriction sites, or by amplifying the genes by PCR. The fragments may contain an intrinsic promoter, or a promoter may be separately cloned. It is advantageous to use a vector carrying a promoter to allow expression of the genes in a *Streptomyces* strain. The plasmid pIJE486 contains a promoter *erm*E for erythromycin resistance gene, allowing constitutive expression of the genes inserted in a correct orientation. Special attention is drawn to the gene encoding a cyclase for the aliphatic ring, but any gene of said cluster may be expressed in *Streptomyces* hosts. The said cyclase converts the stereochemistry at C9 of auramycinone in TK24, if inserted into the plasmid possessing the other genes for auramycinone biosynthesis, except the cyclase responsible for the typical stereochemistry of anthracyclines.

Streptomyces strains, in particular *S. galilaeus*, carrying the recombinant plasmids are cultivated in media wherein antibiotics are produced. The hybrid compounds are extracted with organic solvents from the culture broth, and the compounds are separated and purified using chromatographic techniques.

According to this invention *S. galilaeus* H039 carrying the plasmid pSY42 and designated as H039/pSY42 produces aklavinone-4'-epi-2-deoxyfucose in E1 medium supplemented with thiostrepton to give selection pressure for the plasmid containing strains.

S. lividans TK24 carrying the plasmid pSY15c containing the genes for the nogalamycin chromophor and the genes for a cyclase (*sno*aL) and a ketoreductase (*sno*aF), was cultivated in E1 medium supplemented with thiostrepton. The compound 9-epi-auramycinone was produced, and this structure is now called nogalamycinone. Any DNA fragment of the invention subcloned from a 17 kb nogalamycin biosynthesis region can be inserted in a vector replicating in *Streptomyces*, and the products may be produced by fermentation of the plasmid containing strains.

Brief description of the drawings

Fig. 1 shows the structures of nogalamycin, daunomycin and aclacinomycin.

Fig. 2 is a diagram of the gene cluster (*Sno*5) of the invention for nogalamycin biosynthesis.

Fig. 3 describes the proposed biosynthesis pathway for nogalamycin.

Fig. 4 shows a diagram of the plasmid pSY15c. The genes *sno*aL (**aL**) and *sno*aF (**aF**) shown black are inserted in the plasmid pSY15 to give pSY15c. **aL** represents a cyclase *sno*aL and **aF** is for C-7 ketoreductase *sno*aF. pSY15 (WO 96/10581) generates the production of a tricyclic intermediate for nogalamycin biosynthesis in *S. lividans*. The abbreviations **a1**, **a2** and **a3** refer to the

genes *sno*a1, *sno*a2 and *sno*a3, respectively, for minimal PKS. **rA** is the *snor*A gene for an activator, **aB** is the *sno*aB gene for oxygenase, **aC** is the *sno*aC gene for methylase, **aD** is the *sno*aD gene for polyketide ketoreductase and **aE** is the *sno*aE for aromatase. **gF** (the *sno*gF gene) and **gG** (the *sno*gG gene) involved in the deoxyhexose pathway are not functional in the construct. **aph** is an aminoglycoside phosphotransferase gene, and **tsr** is a thiostreptone resistance gene.

Examples to further illustrate the invention are given hereafter.

EXPERIMENTAL

Materials used

Restriction enzymes used were purchased from Promega (Madison, Wisconsin, USA) or Boehringer Mannheim (Germany), and alkaline phosphatase from Boehringer Mann–heim, and used according to the manufacturers' instructions. Proteinase K was purchased from Promega and lysozyme from Sigma (St. Louis, USA). Hybond™–N nylon membranes used in hybridization were purchased from Amersham (Buckinghamshire, England), DIG DNA Labelling Kit and DIG Luminescent Detection Kit from Boehringer Mannheim. Qiaquick Gel Extraction Kit from Qiagen (Hilden, Germany) was used for isolating DNA from agarose.

Bacterial strains and their use

– *Escherichia coli* XL1 Blue MRF' (Stratagene, La Jolla, CA) was used for cloning.

– *Streptomyces nogalater* ATCC 27451; the gene cluster of nogalamycin biosynthesis was cloned from this strain.

The host strains to express the genes cloned were:

– *Streptomyces lividans* TK24, also used as a primary host to clone DNA propagated in *E. coli*. The strain was provided by prof. Sir David Hopwood, John Innes Centre, UK.

– *Streptomyces galilaeus* H039, produces aklavinone–rhodinose–rhodinose–rhodinose

– *Streptomyces galilaeus* H026, produces aclacinomycin N, ACMN, (aklavinone–rhodosamine–2–deoxyfucose–rhodinose)

– *Streptomyces galilaeus* H063, produces aklavinone

– *Streptomyces galilaeus* H075, produces aklavinone–rhodosamine–2–deoxyfucose–2–deoxyfucose

The detailed description of the mutants H039 and H026 is given in Ylihonko *et al.* (1994) and of H075 in the FI patent application No. 981062 (Ylihonko *et al.*, 1998). H063 has not been described in the literature but it was obtained by NTG mutagenesis of *S. galilaeus*, and selected to be used as the host strain in the hybrid compound production, as it accumulates aklavinone without any sugar residues.

Plasmids

E. coli – *Streptomyces* shuttle cosmid pFD666 (ATCC 77286) was used for cloning the chromosomal DNA. *E. coli* cloning vectors pSL1190 (Pharmacia) and pUC19 were used for preparing the subclones.

pIJ486 is a high copy plasmid vector provided by prof. Sir David Hopwood, John Innes Centre, UK (Ward *et al.*, 1986)

pIJE486 is a vector containing *erm*E gene in the polylinker of pIJ486 (Bibb *et al.*, 1985).

pSY15 is a pIJ486 based plasmid construct, wherein the genes of polyketide pathway for nogalamycin biosynthesis were cloned (Ylihonko *et al.*, 1996a).

Nutrient media and solutions

For cultivation of *S. nogalater* for total DNA isolation TSB medium was used. Lysozyme solution (0.3 M sucrose, 25 mM Tris, pH 8 and 25 mM EDTA pH 8) was used in isolation of total DNA. TE buffer (10 mM Tris, pH 8.0 and 1mM EDTA) was used to dissolve the DNA.

TRYPTONE–SOYA BROTH (TSB)

Per litre: Oxoid Tryptone Soya Broth powder 30 g.

ISP4

Bacto ISP-medium 4, Difco; 37 g/l.

E1 Per litre in tap water:

glucose	20 g
soluble starch	20 g
Farmamedia	5 g
yeast extract	2.5 g
$K_2HPO_4 \bullet 3H_2O$	1.3 g
$MgSO_4 \bullet 7H_20$	1 g
NaCl	3 g
$CaCO_3$	3 g

pH adjusted to 7.4 before autoclaving

General methods

NMR data was collected with a JEOL JNM-GX 400 spectrometer at the ambient temperature. ^1H and ^{13}C NMR samples were internally referenced to TMS.

The anthracycline metabolites were detected by HPLC (LaChrom, Merck Hitachi, pump L-7100, detector L-7400 and integrator D-7500) using a LiChroCART RP-18 column (4.6x250mm). Acetonitrile:potassium hydrogen phosphate buffer (60 mM, pH 3.0 adjusted with citric acid) was used as the mobile phase. Gradient system starting from 65% to 30% of potassium dihydrogen phosphate buffer was used to separate the compounds. The flow rate was 1 ml/min and the detection was effected at 430 nm.

ISP4 plates supplemented with thiostrepton (50 μg/ml) were used to maintain the plasmid carrying cultures.

Example 1. Cloning the gene cluster for nogalamycin biosynthesis

1.1 Cosmid library

For the isolation of total DNA, *Streptomyces nogalater* (ATCC 27451) was grown for three days in 50 ml of TSB medium supplemented with 0.5% of glycine. The cells were harvested by centrifuging for 15 min at 3900 x g in 12 ml Falcon tubes, and the

cells were stored at −20°C. Cells from a 12 ml sample of the culture were used to isolate the DNA. 5 ml of lysozyme solution containing 5 mg of lysozyme/ml was added onto the cells, incubated for 20 min at 37°C. 500 μl of 10% SDS containing 0.7 mg of proteinase K was added onto the cells and incubated for 80 min at 62°C, another 500 μl of 10% SDS containing 0.7 mg of proteinase K was added, and incubation was continued for 60 min. The sample was chilled on ice and 600 μl of 3M NaAc, pH 5.8 were added, and the mixture was extracted with equilibrated phenol (Sigma). The phases were separated by centrifuging at 1400 x g for 10 min. The DNA was precipitated from the water phase with equal volume of isopropanol to spool with a glass rod, and washed by dipping to 70% ethanol, air dried and dissolved in 500 μl of TE−buffer.

The chromosomal DNA was partially digested with *Sau*3AI. The DNA fragments were separated by agarose gel electrophoresis, and the fragments of 30 to 50 kb were cut from the 0.3% low gelling temperature SeaPlaque® agarose. The DNA bands were isolated from the gel by heating to 65°C, extracting with equal volume of equilibrated phenol, and the phases were separated by centrifuging for 15 min at 2500 x g. The phenol phase was extracted with TE buffer, centrifuged and the water phases were pooled. The DNA was precipitated by adding 0.1 volumes of NaAc, pH 5.8 and 2 volumes of ethanol at −20°C for 30 min, centrifuged for 30 min at 15 000 rpm in Sorvall RC5C centrifuge using SS−34 rotor with adapters for 10 ml tubes. The pellet was air dried and dissolved in 20 μl of TE buffer. The isolated fragments were ligated to pFD666 cosmid vector digested with *Bam*HI and dephosphorylated. The DNA was packed into phage particles, and infected to *E. coli* using Gigapack® III XL Packing Extract Kit according to the manufacturer's instructions.

1.2 Identification of the clones by hybridization

The infected cells were grown on LB plates containing 50 μg/ml kanamycin and transferred to Hybond™−N nylon membranes (Amersham). The membranes were handled according to the protocol described in Boehringer Mannheim's manual "The DIG System User's Guide for Filter Hybridization". The probe used to screen the colonies for an expanded nogalamycin gene cluster was a 1.07 kb *Sac*I fragment from the cluster described earlier (Torkkell *et al.*, 1997). The plasmid carrying the probe was

digested with *Sac*I, and the fragment was separated from the vector by agarose gel electrophoresis and isolated from the gel using Qiaquick Gel Extraction Kit (Qiagen). The probe was labelled by digoxygenin using random prime labelling system according to Boehringer Mannheim's manual "The DIG System User's Guide for Filter Hybridization". 5000 colonies were screened by hybridization at 70°C using the probe described. Positive colonies were detected using DIG Luminescent Detection Kit (Boehringer Mannheim). Seven colonies gave a positive signal. Cosmids from the positive clones were isolated from a 5ml culture by alkaline lysis method. Restriction analysis showed that the cloned fragments overlapped each other representing at least 60 kb of the continuous DNA. The positive clones obtained were designated as pFD*Sno*1 to pFD*Sno*7.

1.3. Subcloning the fragments for sequencing

Clone No. 5, designated as pFD*Sno*5, was digested with *Bgl*II, and for subcloning two fragments of about 10 kb and 7 kb were isolated and ligated to pSL1190 digested with *Bgl*II and dephosphorylated. The plasmids obtained were named as pSn42 and pSn43, respectively. These two fragments cover the DNA region flanked to the previously characterized area of nogalamycin biosynthesis cluster. To determine the nucleotide sequence of the whole 17 kb region cloned in pSn42 and pSn43 the convenient restriction sites were used to subclone the fragments to the vector pUC19 or pSL1190 giving 16 subclones from the insert of pSn42 and 11 subclones of pSn43.

E. coli XL1 Blue MRF' cells were cultivated overnight at 37 °C in 5 ml of LB-medium supplemented with 50 μg/ml of ampicillin. To isolate plasmids for sequencing reactions Wizard Plus Minipreps DNA Purification System kit of Promega, or Biometra silica spin plasmid miniprep kit of Biomedizinische Analytik Gmbh were used according to the manufacturers' instructions.

DNA sequencing was performed using the automatic ABI DNA sequenator (Perkin-Elmer) according to the manufacturer's instructions.

1.4 Sequence analysis and the deduced functions of the genes

Sequence analyses were effected using the GCG sequence analysis software package (Version 8; Genetics Computer Group, Madison, Wisconsin, USA). The translation table was modified to accept also GTG as a start codon. Codon usage was analysed using published data (Wright and Bibb 1992).

According to the CODONPREFERENCE program the sequenced DNA fragment (SEQ ID NO:1) contained 15 complete open reading frames (ORFs), and the 5' end of other two ORFs in the both ends of the fragment according to the invention. The functions of the genes were concluded by comparing the amino acid sequences translated from their base sequences to the known protein sequences in the data banks. The results are shown in Table 1. The positions given refer to the appended sequence listing. The amino acid sequences of the peptides are given in SEQ ID NO:2 to SEQ ID NO:18.

Table 1

Gene	Position	Amino acids (SEQ ID NO)	Deduced function	Remarks
*snog*I	−1027 compl	>342 (2)	aminotransferase	5' end
*snog*J	1192–2073	293 (3)	dTDP–glucose synthase	
*snog*A	2106–2822 compl	238 (4)	aminomethyl transferase	
*snoa*M	2826–3800 compl	324 (5)	a polyketide cyclase	
*snog*N	3799–5025	408 (6)	*dnr*Q homology (Otten *et al.*, 1995), unknown	
*snoa*G	5088–6356	422 (7)	hydroxylase	
*snog*C	6334–7209 compl	291 (8)	dTDP–4–dehydrorhamnose reductase	
*snog*K	7245–8297 compl	350 (9)	dTDP–glucose–4,6–de–hydratase	
*snoa*L	8537–8941	134 (10)	NAME cyclase (nogalonic acid methyl ester)	
*sno*K	8992–9699	235 (11)	unknown	
*snog*D	9745–10917 compl	390 (12)	glycosyl transferase	
*sno*W	11057–11884	275 (13)	unknown	
*snog*E	11928–*	>424 (14)	glycosyl transferase	
*sno*L	13335–13754 compl	139 (15)	unknown	
*sno*O	13974–14441	155 (16)	homologous to *mtm*X of mithramycin cluster	
*snoa*F	14532–15377	281 (17)	C–7 ketoreductase analog–ous to aklaviketone keto–reductase	
*sno*N	15450–	>190 (18)	unknown	5' end

*: nucleotide sequence of about 100 bp, not known

1.5 Expression cloning

The 10 kb *Bgl*II fragment from pFD*Sno*5 was cloned into the plasmid pIJ486 and the plasmid obtained was named as pSY42. Correspondingly, the 7 kb *Bgl*II fragment from pFD*Sno*5 was cloned into the plasmid pIJE486, and the plasmid pSY43 was obtained. Plasmid pSY42 was introduced into *S. lividans* strain TK24 by protoplast transformation, isolated from it and further introduced into *S. galilaeus* mutant H039, and after propagation in H039, transferred to other *S. galilaeus* mutants blocked in the deoxyhexose pathway for characteristic sugars of aclacinomycins (H075, H026, and H063). E1 medium was used for anthracycline production, and the products were extracted from the culture with toluene:methanol (1:1) at pH 7. Anthracycline metabolites were analyzed by HPLC. The products of the mutants H039, H026, H063 and H075 carrying pSY42 differed from those obtained by the mutants without the plasmid.

According to the sequence analysis pSY42 contained a cyclase designated as NAMEC (nogalonic acid methyl ester cyclase), and in pSY43 a ketoreductase gene was identified. Expression constructions were prepared which contained all the genes needed for the formation of nogalamycin aglycone. A 1.4 kb *Bam*HI–*Sac*I fragment from pSY42 (containing NAMEC) and a 1.1 kb *Mlu*I–*Kpn*I fragment from pSY43 carrying the gene for a ketoreductase of C–7 keto group were ligated to pSY15 linearized by *Sac*I, to form the plasmid pSY15c (Fig. 4). Plasmid pSY15c was introduced into *S. lividans* TK24, and the strain TK24/pSY15c was cultivated in E1 medium supplemented with thiostrepton. An aglycone compound was produced, and this structure is now called nogalamycinone.

Example 2. Compounds generated by the *sno*5–cluster

2.1 Production and purification of the products derived from H039/pSY42 and TK24/pSY15c

The seed culture, 180 ml of E1 culture of the plasmid containing strain, H039/pSY42 or TK24/pSY15c, was obtained by cultivating the strain in three 250 ml Erlenmeyer flasks containing 60 ml of E1 medium supplemented with thiostrepton (5 μg/ml) for four days at 30°C, 330 rpm. The combined culture broths (180 ml) were used to inoculate 13 l of

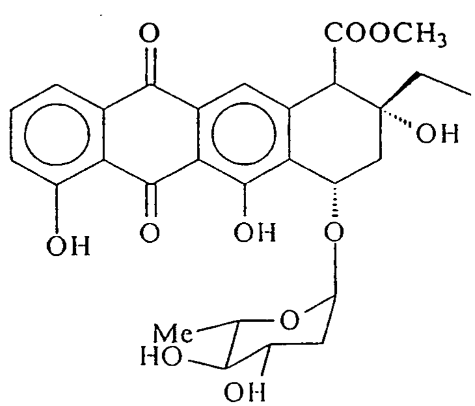
E1 medium in a fermentor (Biostat E). Fermentation was carried out for seven days at 28°C (330 rpm, aeration: 450 l/min).

The cells were harvested by centrifuging. 2.6 l of methanol was used to break the bacterial cells and to extract anthracycline metabolites accumulated. The anthracycline metabolites were extracted using 2 l of dichloromethane at pH 6. The organic layer was evaporated to dryness. The viscous residue was flashed through a polyamide (11) column using water:methanol from 1:9 to 0:10 as the eluent. Pooled fractions containing the compounds were further purified on a Merck–Hitachi HPLC using preparative reversed phase column (LichroCART RP–18, 5 μm) with mobile phase acetonitrile:1 % AcOH in water (1:1). Evaporation of acetonitrile gave pure products as yellow powders dried under vacuum.

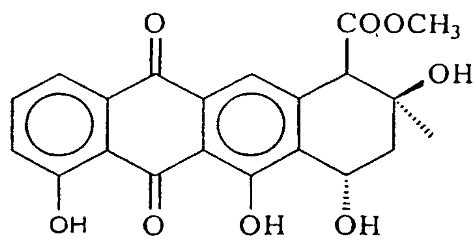
2.2 Structural elucidation of the compounds derived from H039/pSY42 and from TK24/pSY15c

NMR analysis included NON, BMC, NOE, DEPT and HMBC techniques. Protons were assignated using NOESY and 2D pTOCSY techniques and carbons using DEPT and HMBC techniques.

As deduced from the data given in Tables 2 and 3, the structures revealed were aklavinone–4'–epi–2–deoxyfucose from the culture of H039/pSY42, and 9–epi–auramycinone (=nogalamycinone) from the culture of TK24/pSY15c. The chemical structures of the compounds are shown below in Formula I and Formula II, respectively.



(I)



(II)

Deposited microorganisms

The following microorganisms were deposited according to the Budapest Treaty at Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ), Mascheroder Weg 1b, D-38124 Braunschweig, Germany.

Microorganism	**Accession number**	**Date of deposit**
S. lividans TK24/pSY42 carrying the plasmid pSY42	DSM 12451	14 October 1998
S. lividans TK24/pSY43 carrying the plasmid pSY43	DSM 12452	14 October 1998

Table 2. **^{1}H and ^{13}C assignations of the compound aklavinone-4'-epi-2-deoxyfucose (Formula I).**

Site	^{1}H	^{13}C
1	7.74, 1H, dd, 7.5, 1.3	120.1
2	7.68, 1H, dd,. 8.4, 7.5	137.3
3	7.27, 1H, dd, 8.3, 1.3	124.6
4	–	161.9
4–OH	11.70, 1H, s	–
4a	–	115.4
5	–	192.3
5a	–	114.4
6	–	162.4
6–OH	12.46, 1H, s	–
6a	–	130.9
7	5.18, 1H, dd, 4.3, 3.1	71.3
8A	2.51, 1H, dd, 15.0, 4.3	33.9
8B	2.32, 1H, dd, 15.0, 3.1	–
9	–	72.1
9–OH	4.58, 1H, s	–
10	4.02, 1H, s	56.9
10a	–	142.4
11	7.40, 1H, s	120.8
11a	–	133.1
12	–	180.7
12a	–	132.6
13A	1.73, 1H, dq, 14.2, 7.4	32.0
13B	1.51, 1H, dq, 14.2, 7.4	–
14	1.10, 3H, t, 7.4	6.7
15	–	171.1
16	3.69, 3H, s	52.5
1´	5.41, 1H, d, 3.5	101.7
2´a	1.75, 1H, ddd, 12.8, 11.2, 3.4	37.7
2´e	2.19, 1H, dd, 12.8, 5.3	–
3´	3.71, 1H, ddd, 12.0, 9.0, 5.3	69.0
4´	3.14, 1H, dd, 9.1, 9.0	78.1
5´	3.88, 1H, dq, 9.1, 6.2	68.8
6´	1.36, 3H, d, 6.2	17.6

Table 3. ^{1}H and ^{13}C assignations of 9-epi-auramycinone (Formula II).

Site	^{1}H	^{13}C
1	7.76, 1H, dd, 7.5, 1.2	119.8
2	7.67, 1H, dd, 8.3, 7, 5	137.4
3	7.28, 1H, dd, 8.3, 1.2	124.8
4	–	162.5
4–OH	11.86, 1H, s	–
4a	–	115.6
5	–	192.7
5a	–	114.6
6	–	160.9
6–OH	12.76, 1H, s	–
6a	–	134.1
7	5.40, 1H, t, 7.0	64.0
8A	2.66, 1H, dd, 13.9, 7.0	40.9
8B	1.89, 1H, dd, 13.9, 7.1	–
9	–	70.5
9–OH	3.49, 1H, brs	–
10	3.93, 1H, d, 0.8	56.0
10a	–	142.1
11	7.51, 1H, d, 0.8	120.1
11a	–	133.3
12	–	180.9
12a	–	132.1
13	1.44, 3H, s	28.7
14	–	173.0
15	3.90, 3H, s	52.6

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Claims

1. Isolated and purified DNA fragment, which is the gene cluster for the anthracycline biosynthetic pathway of the bacterium *Streptomyces nogalater*, being included in a 10kb and a 7kb flanked *Bgl*II fragments of *S. nogalater* genome.

2. The DNA fragment according to claim 1, comprising the nucleotide sequence given in SEQ ID NO:1, or a sequence showing at least 80% homology to said sequence.

3. A recombinant DNA, which comprises the DNA fragment according to claim 1 or 2, cloned in a plasmid replicating in *Streptomyces*.

4. The recombinant DNA according to claim 3, which is the plasmid pSY15c, comprising a 1.4 kb *Bam*HI–*Sac*I fragment from the plasmid pSY42 and a 1.1 kb *Mlu*I–*Kpn*I fragment from the plasmid pSY43.

5. Plasmid pSY42, deposited in *S. lividans* strain TK24/pSY42 with the deposition number DSM 12451.

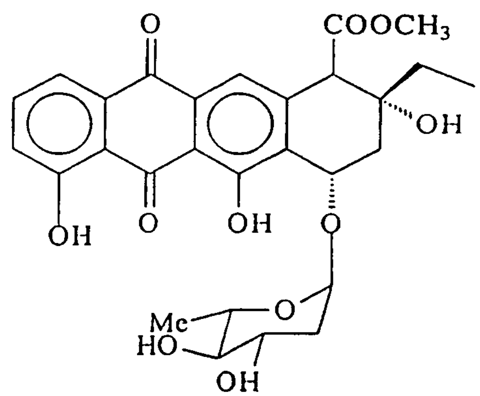
6. Plasmid pSY43, deposited in *S. lividans* strain TK24/pSY43 with the deposition number DSM 12452.

7. A process for the production of hybrid compounds, comprising transferring the DNA fragment according to claim 1 or 2 into a *Streptomyces* host, cultivating the recombinant strain obtained, and isolating the compounds produced.

8. The process according to claim 7, wherein the *Streptomyces* host is a *Streptomyces galilaeus* host.

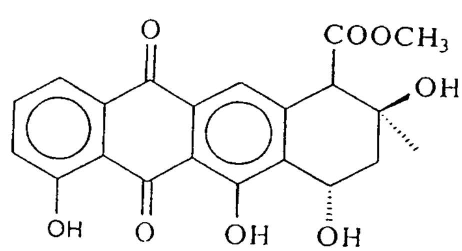
9. The process according to claim 8, wherein the *Streptomyces galilaeus* host is selected from the strains H026, H039, H063 and H075, which are mutant strains of *S. galilaeus* ATCC 31615.

10. The process according to claim 8, wherein an anthracycline is produced, which has the following formula I



(I)

11. The process according to claim 8, wherein an anthracyclinone is produced, which has the following formula II



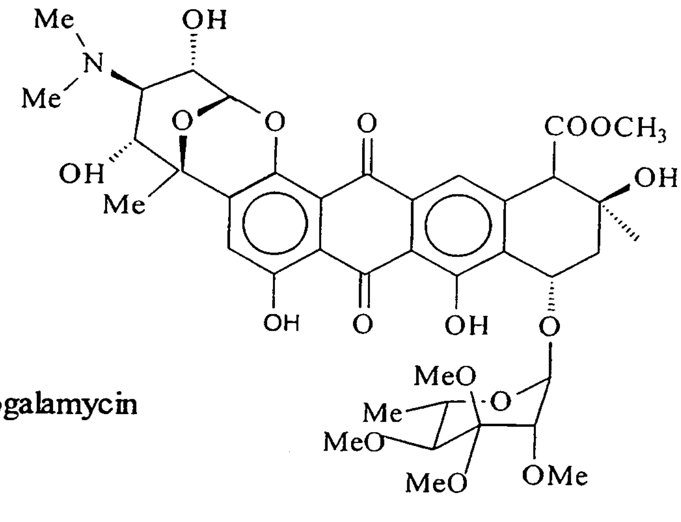
(II)

12. A process for the production of hybrid compounds, comprising transferring at least one of the genes selected from the group consisting of *snog*J, *snog*A, *snoa*M, *snog*N, *snoa*G, *snog*C, *snog*K, *snoa*L, *sno*K, *snog*D, *sno*W, *snog*E, *sno*L, *sno*O and *sno*aF into a *Streptomyces* host, said genes being derived from the DNA fragment of claim 1 or 2, cultivating the recombinant strain obtained, and isolating the compounds produced.

13. The process according to claim 12, wherein the gene *sno*aL encoding NAME cyclase is transferred into a *Streptomyces* host.

14. The process according to claim 12, wherein at least one of the genes *sno*gD and *sno*gE encoding glycosyl transferases is transferred into a *Streptomyces* host.

15. The process according to claim 12, wherein at least one of the genes *sno*gJ, *sno*gN, *sno*gC, *sno*gK and *sno*gA affecting the formation of nogalamine and nogalose is transferred into a *Streptomyces* host.



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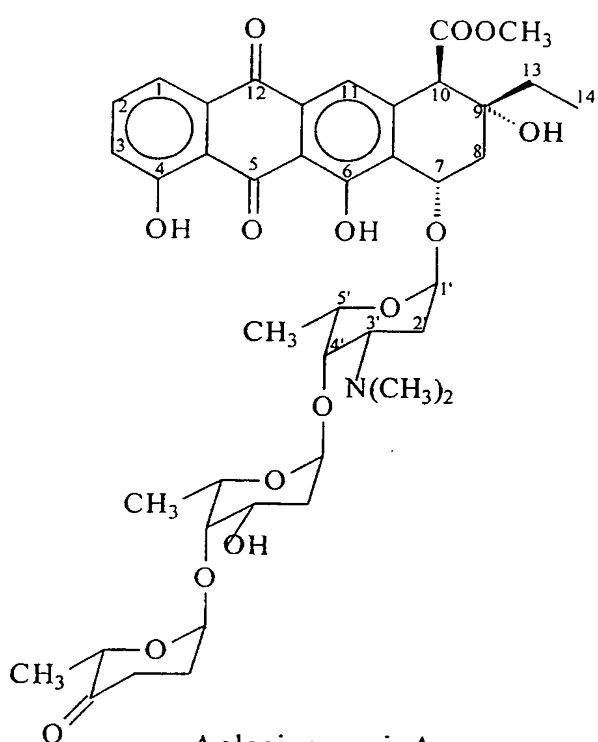

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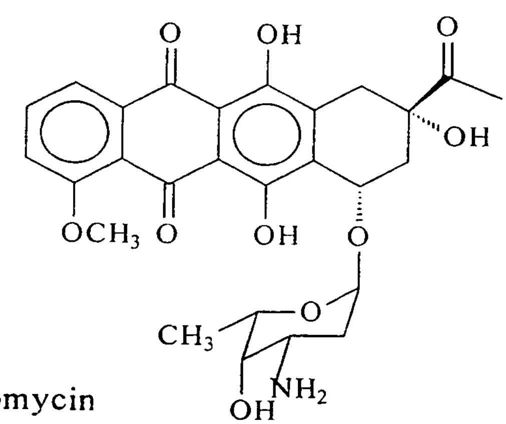
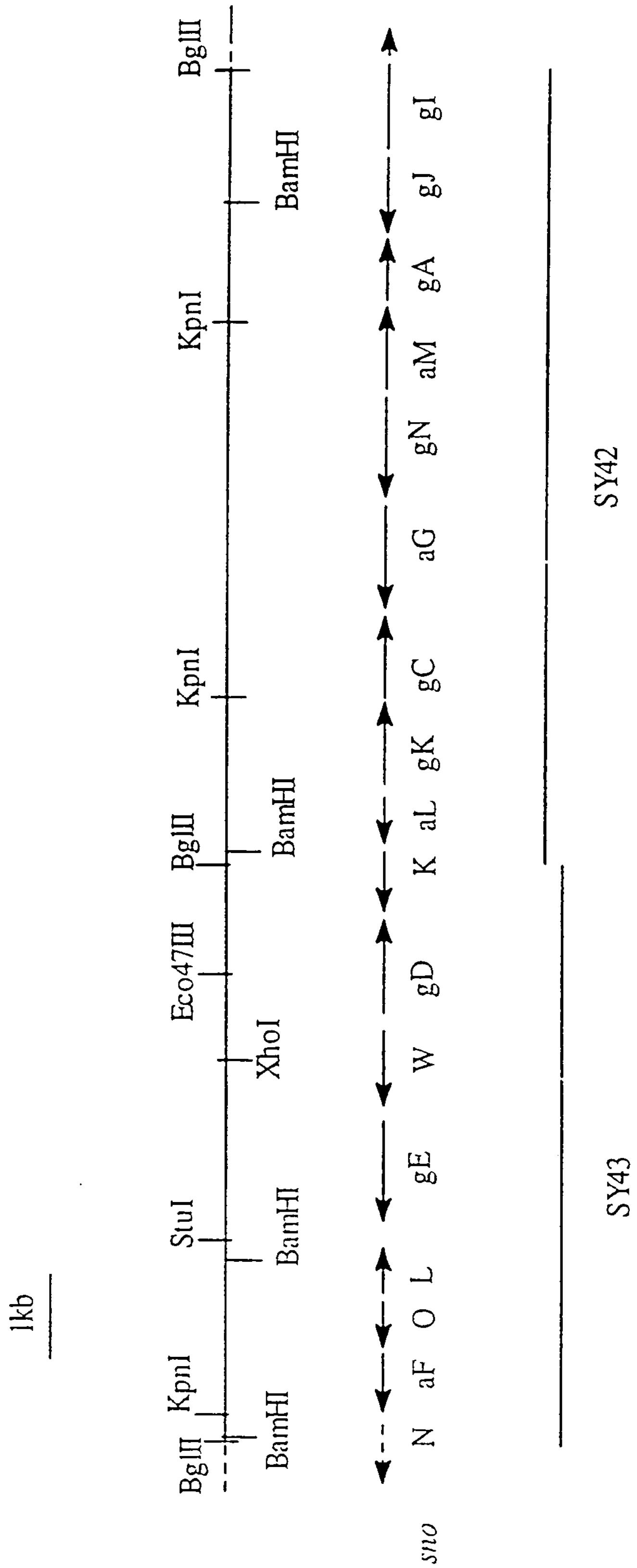


Daunomycin

Fig. 1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aM
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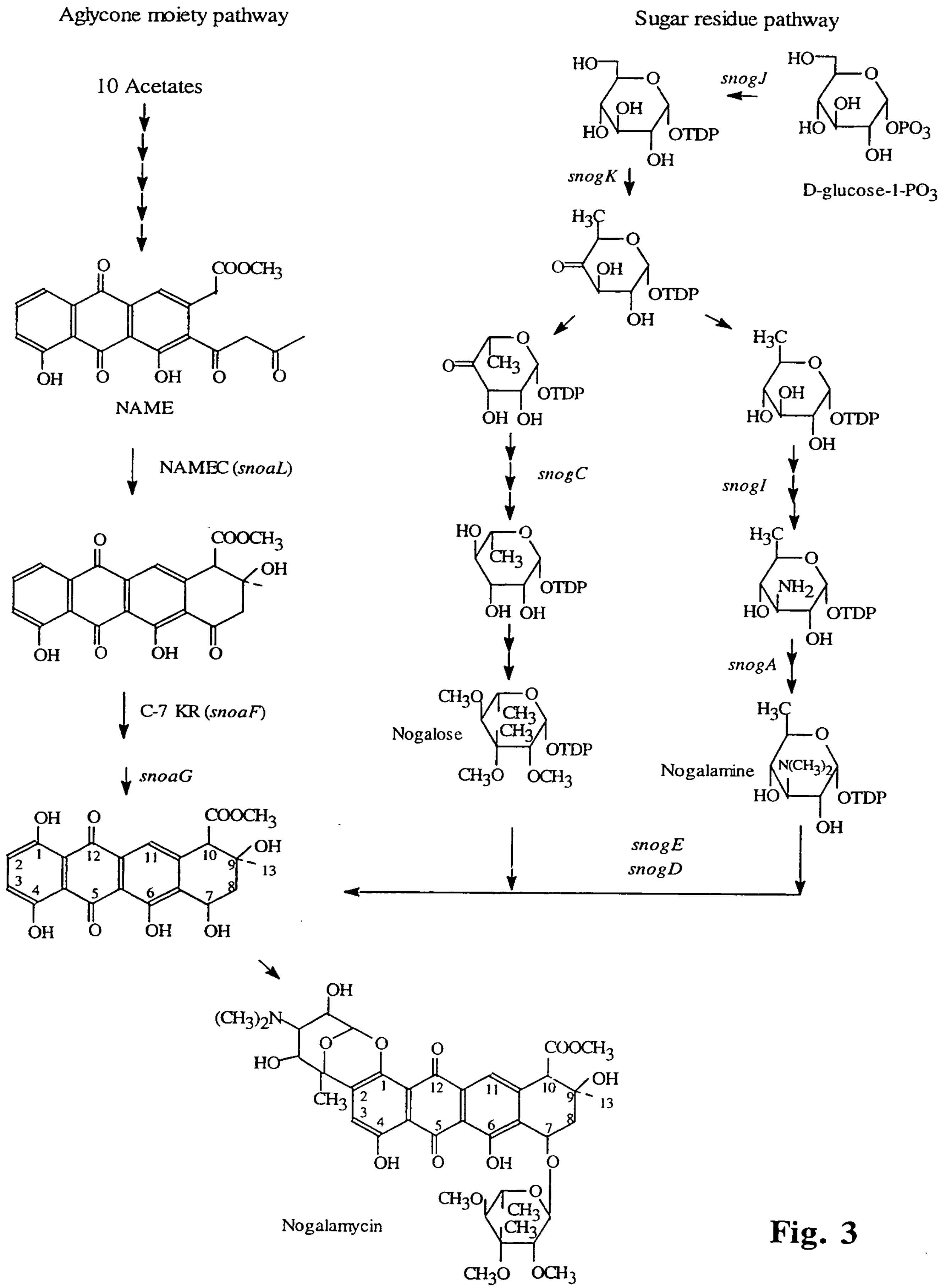
Fig. 2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Sugar residue pathway
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C-7 KR (snoaF)
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Nogalamycin

Fig. 3

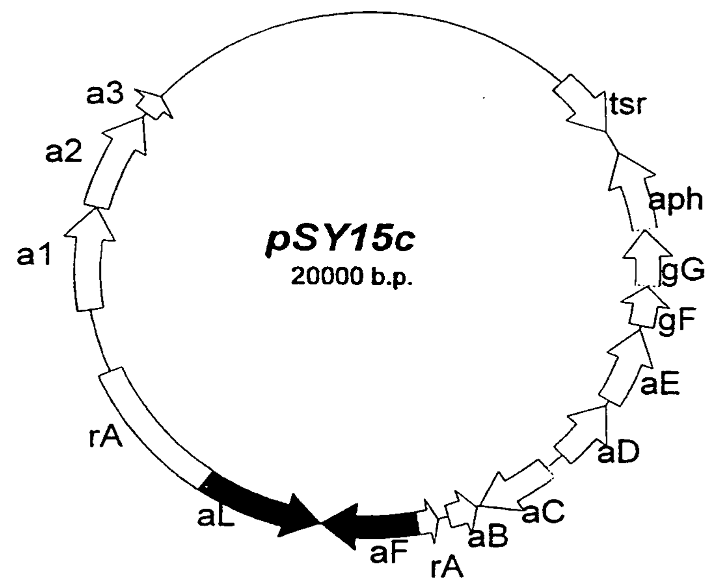

Fig. 4

SEQUENCE LISTING

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<211> 342
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<213> *Streptomyces nogalater* ATCC 27451

<220>
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<400> 2

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Glu Asp Ile His Asp Ala Val Glu Thr Val Phe Arg Ser Gly Arg Leu
20 25 30

Val Leu Gly Glu Ser Val Arg Gly Phe Glu Ser Glu Phe Ala Ser Phe
35 40 45

Gln Gly Val Gly His Ala Val Gly Val Asp Asn Gly Thr Asn Ala Val
50 55 60

Lys Leu Gly Leu Gln Ala Leu Gly Val Gly Pro Gly Asp Glu Val Val
65 70 75 80

Thr Val Ser Asn Thr Ala Ala Pro Thr Val Val Ala Ile Asp Ser Ala
85 90 95

Gly Ala Thr Pro Val Phe Val Asp Val Arg Glu Glu Asp Tyr Leu Met
100 105 110

Asp Thr Ser Gln Val Glu Ala Val Leu Thr Pro Arg Thr Arg Cys Leu
115 120 125

Leu Pro Val His Leu Tyr Gly Gln Cys Val Asp Met Ala Pro Leu Arg
130 135 140

Asp Leu Ala Ala Arg His Asn Leu Val Ile Leu Glu Asp Cys Ala Gln
145 150 155 160

Ala His Gly Ala Arg Arg His Gly Arg Leu Ala Gly Ser Thr Gly Asp
165 170 175

Ala Ala Ala Phe Ser Phe Tyr Pro Thr Lys Val Leu Gly Ala Tyr Gly
180 185 190

Asp Gly Gly Ala Val Leu Thr Asp Asp Glu Arg Val Ala Asp Arg Leu
195 200 205

Arg Arg Leu Arg Tyr Tyr Gly Met Glu Ser Arg Tyr Tyr Val Val Glu
210 215 220

Thr Pro Gly His Asn Ser Arg Leu Asp Glu Val Gln Ala Glu Ile Leu
225 230 235 240

Arg Arg Lys Leu Ser Arg Leu Pro Ser Tyr Ile Glu Ala Arg Arg Ala
245 250 255

Val Ala Arg Arg Tyr Glu Glu Gly Leu Ala Asp Thr Gly Leu Leu Leu
260 265 270

Pro Arg Thr Ala Gln Gly Asn Glu His Val Tyr Tyr Val Tyr Val Val
275 280 285

Arg His Pro Arg Arg Asp Ala Val Leu Glu Ala Leu Arg Ala Ser Tyr
290 295 300

Asp Ile Ala Leu Asn Ile Ser Tyr Pro Trp Pro Val His Thr Met Thr
305 310 315 320

Gly Phe Ser His Leu Gly Tyr Ala Lys Gly Ser Leu Pro Val Thr Glu
325 330 335

Ala Leu Ala Asp Glu Ile
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<210> 3
<211> 293
<212> PRT
<213> *Streptomyces nogalater* ATCC 27451

<220>
<223> "translate of *snogJ*, function: dTDP-glucose synthase"

<400> 3

Val Lys Gly Ile Ile Leu Ala Gly Gly Thr Gly Ser Arg Leu His Pro
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Thr Thr Leu Ala Val Ser Lys Gln Leu Leu Pro Val Gly Asp Lys Pro
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Met Ile Tyr Tyr Pro Leu Ser Val Leu Met Leu Ala Gly Val Thr Asp
35 40 45

Ile Leu Ile Ile Ser Thr Pro His Glu Leu Pro Arg Met Arg Arg Leu
50 55 60

Phe Gly Asp Gly Ala Gln Leu Gly Leu Arg Leu Ala Tyr Ala Glu Gln
65 70 75 80

Glu Lys Pro Arg Gly Ile Ala Glu Ala Phe Leu Ile Gly Ala Asp His
85 90 95

Val Gly Ser Asp Ala Val Ala Leu Ala Leu Gly Asp Asn Ile Phe His
100 105 110

Gly Ser Ser Phe Gln Gly Val Leu Arg Lys Glu Ala Glu Glu Leu Asp
115 120 125

Gly Cys Val Leu Phe Gly Tyr Pro Val Lys Asp Pro Gln Arg Tyr Gly
130 135 140

Val Gly Glu Ala Asn Ala Ser Gly Arg Leu Val Ser Ile Glu Glu Lys
145 150 155 160

Pro Val Arg Pro Arg Ser Asn Arg Ala Ile Thr Gly Leu Tyr Phe Tyr
165 170 175

Asp Asn Glu Val Val Asp Ile Ala Arg Arg Leu Arg Pro Ser Ala Arg
180 185 190

Gly Glu Leu Glu Ile Thr Asp Ile Asn Arg Thr Tyr Met Glu Arg Gly
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Arg Ala Arg Leu Val Asp Leu Gly Arg Gly Phe Ala Trp Leu Asp Thr
210 215 220

Gly Thr Pro Glu Ser Leu Leu Gln Ala Ser Gln Tyr Val Ser Ala Leu
225 230 235 240

Glu Glu Arg Gln Gly Ile Arg Ile Ala Cys Ile Glu Glu Val Ala Leu
245 250 255

Arg Met Gly Phe Ile Asn Ala Gln Ala Cys Tyr Glu Leu Gly Ala Arg
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Cys Thr Gly Arg Val
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<210> 4
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<213> *Streptomyces nogalater* ATCC 27451

<220>
<223> "translate of *snog*A, function: aminomethyl transferase"

<400> 4

Val Tyr Gly Arg Glu Leu Ala Asp Val Tyr Glu Met Val Tyr Arg Ser
1 5 10 15

Arg Gly Lys Ser Trp Ala Asp Glu Ala Glu Arg Val Thr Ala Glu Ile
20 25 30

Arg Ser Arg Arg Pro Gly Ala Arg Ser Leu Leu Asp Val Ala Cys Gly
35 40 45

Thr Gly Ala His Leu Glu Ala Phe Arg Gly Leu Phe Ala His Thr Glu
50 55 60

Gly Leu Glu Leu Ser Asp Glu Met Arg Ala Leu Ala Glu Arg Arg Leu
65 70 75 80

Pro Gly Val Pro Val Arg Pro Gly Asp Met Arg Asp Phe Ala Leu Ser
85 90 95

Gly Arg Phe Asp Ala Val Val Cys Leu Phe Cys Ser Ile Gly Tyr Leu
100 105 110

Glu Thr Val Ala Asp Met Arg Ala Ala Val Arg Thr Met Ala Ala His
115 120 125

Leu Val Pro Gly Gly Val Leu Val Val Glu Pro Trp Trp Phe Pro Glu
130 135 140

Arg Phe Leu Glu Gly Tyr Val Ala Gly Asp Leu Ala Arg Gly Glu Gly
145 150 155 160

Arg Thr Val Ala Arg Val Ser His Ser Thr Arg Gln Gly Arg Arg Thr
165 170 175

Arg Met Glu Val Arg Phe Leu Val Gly Glu Ala Thr Gly Ile Arg Glu
180 185 190

Phe Thr Glu Ile Asp Leu Leu Thr Leu Phe Thr Arg Glu Glu Tyr Leu
195 200 205

Ala Ala Phe Glu Asp Ala Gly Cys Pro Ala Glu Phe Leu Asp Asp Gly
210 215 220

Leu Thr Gly Arg Gly Leu Phe Val Gly Val Arg Gly Ala Gly
225 230 235

<210> 5
<211> 324
<212> PRT
<213> *Streptomyces nogalater* ATCC 27451

<220>
<223> "translate of *sno*aM, function: polyketide cyclase"

<400> 5

Met Thr Ala Ala Trp Gly Ala Pro Leu Tyr Pro Pro Trp Ile Pro Ala
1 5 10 15

Arg Pro Gly Arg Arg Arg Cys Gly Ala Gly Arg Arg Val Arg Cys Pro
20 25 30

Pro Val Glu Pro Ala Ser Arg Pro Arg Gln Glu Gly Arg Val Ser Val
35 40 45

Val Pro Ala Leu Arg Gln Pro Ser Pro Ser Thr Asn Pro Glu Val Arg
50 55 60

Val Arg Leu Ile Asp Leu Ser Ser Pro Val Asp Ser Ser Gln Tyr Glu
65 70 75 80

Pro Asp Pro Val Val His Asp Val Leu Thr Pro Arg Gln Gly Ala Glu
85 90 95

His Met Cys Ala Glu Met Arg Glu His Phe Gly Val Glu Phe Ser Pro
100 105 110

Asp Glu Leu Pro Asp Gly Glu Phe Leu Ser Leu Asp Arg Ile Thr Leu
115 120 125

Thr Thr His Thr Gly Thr His Val Asp Ala Pro Ser His Tyr Gly Ser
130 135 140

Arg Ala Leu Tyr Gly Asp Gly Val Pro Arg His Ile Asp Gln Met Pro
145 150 155 160

Leu Glu Trp Phe Phe Gly Arg Gly Val Val Leu Asp Leu Thr Asp Ala
165 170 175

Pro Thr Gly Thr Val Ser Ala Ala Arg Leu Glu Lys Glu Leu Ala Arg
180 185 190

Thr Gly Cys Ala Leu Arg Pro Gly Asp Ile Val Leu Leu His Thr Gly
195 200 205

Ala Gln Arg His Ala Gly Thr Pro Arg Tyr Phe Thr Asp Phe Ala Gly
210 215 220

Leu Asp Gly Pro Ala Val Arg Met Leu Leu Asp His Gly Val Arg Val
225 230 235 240

Ile Gly Thr Asp Ala Phe Ser Leu Asp Ala Pro Phe Gly His Ile Ile
245 250 255

Asp Arg Tyr Arg Ala Thr Gly Asp Arg Ser Val Leu Trp Pro Ala His
260 265 270

Val Val Gly Arg Glu Arg Glu Tyr Cys Gln Ile Glu Arg Leu Ala Asn
275 280 285

Leu Asp Arg Leu Pro Val Ser Phe Gly Phe Arg Val Cys Cys Phe Pro
290 295 300

Val Lys Val Ala Gly Ala Gly Ala Gly Trp Thr Arg Ala Val Ala Leu
305 310 315 320

Val Asp Glu Asp

<210> 6
<211> 408
<212> PRT
<213> *Streptomyces nogalater* ATCC 27451

<220>
<223> "translate of *snog*N, function: unknown"

<400> 6

Met Val Met Lys Leu Thr Asp Ser Glu Leu Gly Arg Ala Leu Leu Ser
1 5 10 15

Leu Arg Gly Tyr Gln Trp Leu Arg Gly Ile His His Asp Pro Tyr Ala
20 25 30

Leu Leu Leu Arg Ala Glu Ser Asp Asp Pro Ala Gln Leu Gly Arg Leu
35 40 45

Leu Arg Glu Arg Gly Arg Leu His Arg Ser Asp Thr Gly Thr Trp Val
50 55 60

Thr Ala Asp His Ala Thr Ala Ser Arg Leu Leu Ala Asp Pro Arg Phe
65 70 75 80

Val Leu Arg Arg Pro Pro Ala Gly Pro Ala Thr Gly Thr Gly Asp Val
85 90 95

Met Pro Trp Glu Glu Ala Thr Leu Ser Asp Leu Leu Pro Leu Asp Glu
100 105 110

Ala Arg Leu Thr Thr Asp Arg Ala Arg Cys Arg Arg Leu Gly Ala Thr
115 120 125

Ala Ala Arg Ile Ala Ala Asp Gly Pro Val Ala Thr Arg Leu Ala Asp
130 135 140

Leu Ala Gly Ala Arg Ala Glu Gln Val Arg Ser Thr Gly His Phe Asp
145 150 155 160

Leu Arg Ala Asp Tyr Ala Leu Pro Tyr Ala Val Glu Pro Ala Cys Ala
165 170 175

Leu Leu Gly Leu Pro Ala Gly Gln Cys Ser Leu Phe Gly Ala Phe Ser
180 185 190

Pro Ala Val Leu Leu Asp Ala Thr Val Val Pro Pro Arg Leu Pro Glu
195 200 205

Ala Arg Ala Leu Ile Ala Ser Thr Ala Glu Leu Thr Ala Leu Trp Pro
210 215 220

Arg Leu Ala Pro Ser Leu Ser Lys Thr Val Pro Glu Asp Glu Ala Pro
225 230 235 240

Asp Leu Phe Leu Leu Thr Ala Val Leu Leu Val Pro Ala Val Val His
245 250 255

Leu Val Cys Glu Ala Val Ala Ala Leu Ser His Asp Pro Gly Gln Ala
260 265 270

Gly Leu Leu Arg Asp Asp Pro Val Leu Ala Ala Pro Ala Val Glu Glu
275 280 285

Thr Leu Arg His Ala Pro Pro Ala Arg Leu Phe Thr Leu His Ala Thr
290 295 300

Gly Pro Glu Arg Val Ala Asp Val Asp Leu Pro Ala Gly Ala Glu Val
305 310 315 320

Ala Val Val Val Ala Ala Ala His Arg Asp Pro Ser Trp Cys Pro Asp
325 330 335

Pro Asp Arg Phe Asp Leu Thr Arg Asn Glu Arg His Leu Ala Leu Pro
340 345 350

Pro Asp Leu Pro Leu Gly Ala Leu Ala Pro Leu Leu Arg Val Cys Ala
355 360 365

Thr Ala Ala Val Ala Ala Leu Ala Ala Gly Leu Leu Pro Leu Arg Ala
370 375 380

Val Gly Pro Pro Val Arg Arg Leu Arg Ala Pro Val Thr Arg Ser Val
385 390 395 400

Leu Arg Phe Pro Val Ala Pro Cys
405

<210> 7
<211> 422
<212> PRT
<213> *Streptomyces nogalater* ATCC 27451

<220>
<223> "translate of *sno*aG, function: hydroxylase"

<400> 7

Met Asp Asn Arg Glu Thr Val Arg Pro Val Ser Val Cys Arg Val Cys
1 5 10 15

Gly Gly Asn Asp Trp Gln Asp Val Val Asp Phe Gly Asp Val Pro Leu
20 25 30

Ala Asn Gly Phe Leu Ser Pro Ala Asp Ser Tyr Glu Asn Glu Arg Arg
35 40 45

Tyr Pro Leu Gly Val Leu Ser Cys Arg Ala Cys Arg Leu Met Ser Leu
50 55 60

Thr His Val Val Asp Pro Glu Val Leu Tyr Arg Asp Tyr Ala Tyr Thr
65   70   75   80

Thr Pro Asp Ser Glu Met Ile Thr Gln His Met Arg His Ile Thr Ala
                85   90   95

Leu Cys Arg Thr Arg Phe Glu Leu Pro Pro Asp Ser Leu Val Val Glu
            100   105   110

Leu Gly Ser Asn Thr Gly Arg Gln Leu Met Ala Phe Arg Glu Ala Gly
        115   120   125

Met Arg Thr Leu Gly Val Asp Pro Ala Arg Asn Leu Thr Asp Val Ala
    130   135   140

Arg Arg Asn Gly Ile Glu Thr Phe Pro Asp Phe Phe Ser His Asp Val
145   150   155   160

Ala Arg Thr Ile Arg Arg Asp His Gly Gln Ala Arg Leu Val Leu Gly
                165   170   175

Arg His Val Phe Ala His Ile Asp Asp Val Ser Asp Ile Ala Ala Gly
            180   185   190

Val Arg Glu Leu Leu Ser Pro Asp Gly Val Phe Ala Ile Glu Val Pro
        195   200   205

Tyr Val Leu Asp Leu Leu Glu Lys Val Ala Phe Asp Thr Ile Tyr His
    210   215   220

Glu His Leu Ser Tyr Phe Thr Met Arg Ser Phe Val Thr Leu Phe Ala
225   230   235   240

Arg His Gly Leu Arg Val Leu Asp Val Glu Arg Phe Gly Val His Gly
                245   250   255

Gly Ser Val Leu Val Phe Val Gly His Glu Asp Gly Pro Trp Pro Glu
            260   265   270

Arg Pro Ser Val Pro Glu Leu Leu Arg Val Glu Arg Gln Arg Gly Leu
        275   280   285

Tyr Asp Asp Ala Thr Tyr Arg Thr Phe Ala Gln Arg Ile Glu Arg Val
    290   295   300

Arg Thr Glu Leu Pro Glu Leu Leu Arg Ser Leu Val Ala Gln Gly Lys
305   310   315   320

Arg Ile Val Gly Tyr Gly Ala Pro Ala Lys Gly Asn Thr Ile Leu Thr
                325   330   335

Val Cys Gly Leu Gly Leu Lys Glu Leu Glu Tyr Cys Thr Asp Thr Thr
            340   345   350

Glu Leu Lys Gln Gly Arg Val Leu Pro Gly Thr His Ile Pro Val His
        355   360   365

Ala Pro Glu His Ala Lys Glu His Ile Pro Asp Tyr Tyr Leu Leu Leu
    370   375   380

Ala Trp Asn Tyr Ala Thr Glu Ile Leu Asp Lys Glu Thr Ala Phe Arg
385   390   395   400

Asp Asn Gly Gly Arg Phe Ile Val Pro Ile Pro Arg Pro Ser Ile Leu
                405   410   415

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Thr Ser Pro Ser Gly Ser
            420

<210>   8
<211>   291
<212>   PRT
<213>   Streptomyces nogalater ATCC 27451

<220>
<223>   "translate of snogC, function: dTDP-4-dehydrorhamnose reductase"

<400>   8

Met Leu Ala Arg His Leu Thr Ala Ala Leu Ala Glu Thr Gly Arg Ser
1               5                   10                  15

Arg Pro Ala Ala Glu Ala Val Val Leu Gly Arg Arg Ala Leu Asp Ile
            20                  25                  30

Thr Asp Gly Arg Ala Val Asp Ala Ala Phe Ala Ala His Arg Pro Arg
        35                  40                  45

Val Val Val Asn Cys Ala Ala Phe Thr Asp Val Asp Gly Ala Glu Ser
    50                  55                  60

Arg Trp Ala Glu Ala Met Arg Val Asn Gly Gly Gly Pro Arg Leu Leu
65                  70                  75                  80

Ala Arg Arg Cys Ala Arg His Gly Val Arg Leu Ile His Val Ser Thr
                85                  90                  95

Asp Tyr Val Phe Pro Gly Asp Thr Arg Ser Pro Tyr Gly Glu Ser Asp
            100                 105                 110

Ala Pro Gly Pro Arg Thr Val Tyr Gly Arg Ser Lys Leu Ala Gly Glu
        115                 120                 125

Arg Ala Val Leu Ser Leu Leu Pro Asp Thr Gly Thr Val Val Arg Thr
    130                 135                 140

Ala Trp Leu Tyr Gly Gly Gln Gly Arg Ser Phe Val Arg Thr Met Leu
145                 150                 155                 160

Glu Arg Ala Pro Asp Asp Gly His Val Asp Val Val Asn Asp Gln Trp
                165                 170                 175

Gly Gln Pro Thr Trp Ala Gly Asp Val Ala Arg Leu Leu Val Thr Leu
            180                 185                 190

Ala Arg Thr Pro Pro Asp Arg Ala Arg Gly Ile Phe His Ala Thr Asn
        195                 200                 205

Ala Gly Ala Ala Thr Trp Tyr Glu Leu Ala Arg Glu Val Phe Arg Leu
    210                 215                 220

Ala Gly Ala Asp Pro Glu Arg Val Arg Pro Val Ala Thr Ala Asp Arg
225                 230                 235                 240

Pro Gly Pro Ala Pro Arg Pro Ala Cys Thr Val Leu Gly His Asp Arg
                245                 250                 255

Trp Arg Leu Val Gly Val Ala Pro Pro Arg Asp Trp Arg Ala Ala Leu
            260                 265                 270

Arg Glu Ala Met Arg Gln Leu Leu Pro Gly Gly Arg Leu Arg Asn Leu
        275                 280                 285
```

Thr Gly Thr
290

<210> 9
<211> 350
<212> PRT
<213> *Streptomyces nogalater* ATCC 27451

<220>
<223> "translate of *snog*K, function: dTDP-glucose-4,6-dehydratase"

<400> 9

Met Ala Ser His Thr Ser Ala Thr Thr Asp Val Asn Ile Leu Val Thr
1 5 10 15

Gly Ala Val Gly Phe Ile Gly Ser Ala Tyr Val Arg Met Leu Leu Glu
20 25 30

Asn Arg Ala Pro Gly Ala Gly Ala Pro Ala Val Arg Val Thr Val Leu
35 40 45

Asp Lys Leu Thr Tyr Ala Gly Asn Leu Thr Asn Leu Asp Ala Val Arg
50 55 60

Gly Asp Arg Leu Arg Phe Val Arg Gly Asp Ile Leu Asp Ala Glu Leu
65 70 75 80

Val Asp Glu Leu Met Ala His Ser Asp Gln Val Val His Phe Ala Ala
85 90 95

Glu Ser His Val Asp Arg Ser Ile Arg Ala Ala Asp Asp Phe Val Leu
100 105 110

Thr Asn Val Val Gly Thr Gln Arg Leu Leu Asp Ala Ala Leu Arg His
115 120 125

Gly Val Glu Pro Phe Val Leu Val Ser Thr Asp Glu Val Tyr Gly Ser
130 135 140

Ile Ala Ser Gly Ser Trp Pro Glu Glu His Pro Leu Ser Pro Asn Ser
145 150 155 160

Pro Tyr Ala Ala Ser Lys Ala Ser Ala Asp Leu Met Ala Phe Ala Cys
165 170 175

His Arg Thr His Gly Leu Asp Val Arg Val Thr Arg Cys Ser Asn Asn
180 185 190

Tyr Gly Pro Arg Gln His Pro Glu Lys Leu Ile Pro Arg Phe Val Thr
195 200 205

Asn Leu Leu Asp Gly Leu Pro Val Pro Leu Tyr Gly Asp Gly Arg Asn
210 215 220

Val Arg Glu Trp Leu His Val Glu Asp His Cys Arg Gly Val Asp Leu
225 230 235 240

Val Arg Thr Ala Gly Arg Pro Gly Gly Val Tyr His Ile Gly Gly Gly
245 250 255

Arg Glu Leu Ser Asn Arg Glu Leu Val Gly Met Leu Leu Glu Leu Cys
260 265 270

Gly Ala Asp Trp Ser Ser Val Arg His Val Pro Asp Arg Lys Gly His
275 280 285

Asp Leu Arg Tyr Ser Leu Asp Trp Gly Arg Ala Arg Glu Glu Leu Gly
290 295 300

Tyr Arg Pro Ala Arg Glu Phe Ser Ser Gly Leu Arg Ser Thr Val Gln
305 310 315 320

Trp Tyr Arg Glu Asn Arg Ser Trp Trp Glu Pro Leu Lys Arg Gly Val
325 330 335

Thr Ala Pro Gly Gly Thr Ser Thr Val Val Pro Gly Val Arg
340 345 350

<210> 10
<211> 134
<212> PRT
<213> *Streptomyces nogalater* ATCC 27451

<220>
<223> "translate of *sno*aL, function: NAME cyclase"

<400> 10

Met Val Ser Ala Phe Asn Thr Gly Arg Thr Asp Asp Val Asp Glu Tyr
1 5 10 15

Ile His Pro Asp Tyr Leu Asn Pro Ala Thr Leu Glu His Gly Ile His
20 25 30

Thr Gly Pro Lys Ala Phe Ala Gln Leu Val Gly Trp Val Arg Ala Thr
35 40 45

Phe Ser Glu Glu Ala Arg Leu Glu Glu Val Arg Ile Glu Glu Arg Gly
50 55 60

Pro Trp Val Lys Ala Tyr Leu Val Leu Tyr Gly Arg His Val Gly Arg
65 70 75 80

Leu Val Gly Met Pro Pro Thr Asp Arg Arg Phe Ser Gly Glu Gln Val
85 90 95

His Leu Met Arg Ile Val Asp Gly Lys Ile Arg Asp His Arg Asp Trp
100 105 110

Pro Asp Phe Gln Gly Thr Leu Arg Gln Leu Gly Asp Pro Trp Pro Asp
115 120 125

Asp Glu Gly Trp Arg Pro
130

<210> 11
<211> 235
<212> PRT
<213> *Streptomyces nogalater* ATCC 27451

<220>
<223> "translate of *sno*K, function: unknown"

<400> 11

Met Pro Asp Pro Gly Gly Pro Thr Thr Ala Glu Asn Leu Ser Lys Glu
1 5 10 15

Ala Val Arg Phe Tyr Arg Glu Gln Gly Tyr Val His Ile Pro Arg Val
20 25 30

Leu Ser Glu Thr Glu Val Thr Ala Phe Arg Ala Ala Cys Glu Glu Val
35 40 45

Leu Glu Lys Glu Gly Arg Glu Ile Ser Gly Ile Ala Leu Arg Leu Ala
50 55 60

Gly Ala Pro Leu Arg Val Tyr Ser Ser Asp Ile Leu Val Lys Glu Pro
65 70 75 80

Lys Arg Thr Leu Pro Thr Leu Val His Asp Asp Glu Thr Gly Leu Pro
85 90 95

Leu Asn Glu Leu Ser Ala Thr Leu Thr Ala Trp Ile Ala Leu Thr Asp
100 105 110

Val Pro Val Glu Arg Gly Cys Met Ser Tyr Val Pro Gly Ser His Leu
115 120 125

Arg Ala Arg Glu Asp Arg Gln Glu His Met Thr Ser Phe Ala Glu Phe
130 135 140

Arg Asp Leu Ala Asp Val Trp Pro Asp Tyr Pro Trp Gln Pro Arg Val
145 150 155 160

Ala Val Pro Val Arg Ala Gly Asp Val Val Phe His His Cys Arg Thr
165 170 175

Val His Met Ala Glu Ala Asn Thr Ser Asp Ser Val Arg Met Ala His
180 185 190

Gly Val Val Tyr Met Asp Ala Asp Ala Thr Tyr Arg Pro Gly Val Gln
195 200 205

Asp Gly His Leu Ser Arg Leu Ser Pro Gly Asp Pro Leu Glu Gly Glu
210 215 220

Leu Phe Pro Leu Val Thr Ala Gly Thr Arg Gln
225 230 235

<210> 12
<211> 390
<212> PRT
<213> *Streptomyces nogalater* ATCC 27451

<220>
<223> "translate of *snog*D, function: glycosyl transferase"

<400> 12

Met Arg Val Pro Gly Ser Cys Arg Thr Gly Gly Ile Met Arg Ala Leu
1 5 10 15

Phe Ile Thr Ser Pro Gly Leu Ser His Ile Leu Pro Thr Val Pro Leu
20 25 30

Ala Gln Ala Leu Arg Ala Leu Gly His Glu Val Arg Tyr Ala Thr Gly
35 40 45

Gly Asp Ile Arg Ala Val Ala Glu Ala Gly Leu Cys Ala Val Asp Val
50 55 60

Ser Pro Gly Val Asn Tyr Ala Lys Leu Phe Val Pro Asp Asp Thr Asp
65 70 75 80

Val Thr Asp Pro Met His Ser Glu Gly Leu Gly Glu Gly Phe Phe Ala
85 90 95

Glu Met Phe Ala Arg Val Ser Ala Val Ala Val Asp Gly Ala Leu Arg
100 105 110

Thr Ala Arg Ser Trp Arg Pro Asp Leu Val Val His Thr Pro Thr Gln
115 120 125

Gly Ala Gly Pro Leu Thr Ala Ala Ala Leu Gln Leu Pro Cys Val Glu
130 135 140

Leu Pro Leu Gly Pro Ala Asp Ser Glu Pro Gly Leu Gly Ala Leu Ile
145 150 155 160

Arg Arg Ala Met Ser Lys Asp Tyr Glu Arg His Gly Val Thr Gly Glu
165 170 175

Pro Thr Gly Ser Val Arg Leu Thr Thr Thr Pro Pro Ser Val Glu Ala
180 185 190

Leu Leu Pro Glu Asp Arg Arg Ser Pro Gly Ala Trp Pro Met Arg Tyr
195 200 205

Val Pro Tyr Asn Gly Gly Ala Val Leu Pro Asp Trp Leu Pro Pro Ala
210 215 220

Ala Gly Arg Arg Arg Ile Ala Val Thr Leu Gly Ser Ile Asp Ala Leu
225 230 235 240

Ser Gly Gly Ile Ala Lys Leu Ala Pro Leu Phe Ser Glu Val Ala Asp
245 250 255

Val Asp Ala Glu Phe Val Leu Thr Leu Gly Gly Gly Asp Leu Ala Leu
260 265 270

Leu Gly Glu Leu Pro Ala Asn Val Pro Val Val Glu Trp Ile Pro Leu
275 280 285

Gly Ala Leu Leu Glu Thr Cys Asp Ala Ile Ile His His Gly Gly Ser
290 295 300

Gly Thr Leu Leu Thr Ala Leu Ala Ala Gly Val Pro Gln Cys Val Ile
305 310 315 320

Pro His Gly Ser Tyr Gln Asp Thr Asn Arg Asp Val Leu Thr Gly Leu
325 330 335

Gly Ile Gly Phe Asp Ala Glu Ala Gly Ser Leu Gly Ala Glu Gln Cys
340 345 350

Arg Arg Leu Leu Asp Asp Ala Gly Leu Arg Glu Ala Ala Leu Arg Val
355 360 365

Arg Gln Glu Met Ser Glu Met Pro Pro Pro Ala Glu Thr Ala Ala Lys
370 375 380

Leu Val Ala Leu Ala Gly
385 390

<210> 13
<211> 275
<212> PRT
<213> *Streptomyces nogalater* ATCC 27451

<220>
<223> "translate of *sno*W, function: unknown"

<400> 13

Met Thr Val Leu Val Thr Gly Ala Thr Gly Asn Val Gly Arg His Val
1 5 10 15

Val Thr Gly Leu Leu Ala Ala Gly Arg Arg Val Arg Ala Leu Thr Arg
20 25 30

Thr Pro Asp Arg Ser Gly Leu Pro Gly Gly Ala Glu Ile Thr Gly Gly
35 40 45

Asp Leu Thr Arg Pro Glu Thr Tyr Glu Arg Met Leu Asp Gly Val Glu
50 55 60

Ala Val Tyr Leu Phe Pro Val Pro Glu Thr Ala Ala Ala Phe Ala Gly
65 70 75 80

Ala Ala Arg Arg Ala Gly Val Arg Arg Ile Val Val Leu Ser Ser Asp
85 90 95

Ser Val Thr Asp Gly Thr Asp Thr Gly Gly His Arg Arg Val Glu Leu
100 105 110

Ala Val Glu Asp Thr Gly Leu Glu Trp Thr His Val Arg Pro Gly Glu
115 120 125

Phe Ala Leu Asn Lys Val Thr Leu Trp Ala Pro Ser Ile Arg Ala Glu
130 135 140

Gly Val Val Arg Ser Ala Tyr Pro Asp Ala Arg Val Ala Pro Val His
145 150 155 160

Glu Ala Asp Val Ala Ala Val Ala Val Thr Ala Leu Leu Lys Glu Gly
165 170 175

His Ala Gly Arg Ala Tyr Ser Val Thr Gly Pro Gln Ala Leu Thr Gln
180 185 190

Arg Glu Gln Val Arg Ala Val Gly Glu Gly Leu Gly Arg Ser Leu Ala
195 200 205

Phe Val Glu Val Thr Pro Gly Gln Ala Arg Ala Asp Leu Thr Ala Gln
210 215 220

Gly Leu Pro Ala Pro Ile Ala Asp Tyr Val Leu Ala Phe Gln Ala Gly
225 230 235 240

Trp Thr Glu Arg Pro Ala Pro Ala Arg Pro Thr Val Arg Glu Val Thr
245 250 255

Gly Arg Pro Ala Arg Thr Leu Ala Gln Trp Ala Ala Asp His Arg Ala
260 265 270

Asp Phe Arg
275

<210> 14
<211> 424
<212> PRT
<213> *Streptomyces nogalater* ATCC 27451

<220>
<223> "translate of *snog*E, function: glycosyl transferase"

<400> 14

Val Arg Val Leu Leu Thr Ser Phe Ala Met Asp Ala His Phe Cys Thr
1 5 10 15

Ala Val Pro Leu Ala Trp Ala Leu Arg Ser Ala Gly His Glu Val Arg
20 25 30

Val Ala Gly Gln Pro Ala Leu Thr Ser Thr Ile Thr Gly Ala Gly Leu
35 40 45

Thr Ala Val Pro Val Gly Arg Asp His Thr His Gly Ser Leu Leu Gly
50 55 60

Arg Val Gly Ser Asp Ile Leu Ala Leu His Asp Glu Ala Asp Tyr Leu
65 70 75 80

Glu Ala Arg His Asp Ala Leu Gly Phe Glu Phe Leu Lys Gly His Asn
85 90 95

Thr Val Met Ser Ala Leu Phe Tyr Ser Gln Ile Asn Asn Asp Ser Met
100 105 110

Val Asp Asp Leu Val Asp Phe Ala Arg His Trp Arg Pro Asp Leu Val
115 120 125

Val Trp Glu Pro Phe Thr Phe Ala Gly Ala Val Ala Ala Arg Ala Ser
130 135 140

Gly Ala Ala His Ala Arg Leu Leu Ser Phe Pro Asp Leu Phe Leu Ser
145 150 155 160

Thr Arg Arg Leu Phe Leu Glu Arg Met Ala Arg Gln Glu Pro Glu His
165 170 175

His Asp Asp Thr Leu Ala Glu Trp Leu Asp Trp Thr Leu Gly Arg His
180 185 190

Gly His Ser Phe Asp Glu Glu Ile Val Thr Gly Gln Trp Ser Ile Asp
195 200 205

Gln Thr Pro Ala Pro Val Arg Leu Asp Ala Gly Gly Pro Thr Val Pro
210 215 220

Met Arg Tyr Val Pro Tyr Ser Gly Leu Val Pro Thr Val Val Pro Asp
225 230 235 240

Trp Leu Arg Arg Pro Pro Glu Arg Pro Arg Val Leu Val Thr Leu Gly
245 250 255

Ile Thr Ser Arg Arg Val Lys Ser Phe Leu Ala Val Ser Val Asp Asp
260 265 270

Leu Phe Glu Ala Val Ala Gly Leu Gly Val Glu Val Val Ala Thr Leu
275 280 285

Asp Ala Asp Gln Arg Glu Leu Leu Gly Arg Val Pro Asp His Phe Arg
290 295 300

Ile Val Glu His Val Pro Leu Asp Ala Val Leu Pro Thr Cys Ser Ala
305 310 315 320

Ile Val His His Gly Gly Ala Gly Thr Trp Ser Thr Ala Ala Val Tyr
325 330 335

Gly Val Pro Gln Val Ser Leu Gly Ser Met Trp Asp His Phe Tyr Arg
340 345 350

Ala Arg Arg Leu Glu Glu Leu Gly Ala Gly Leu Arg Leu Pro Ser Gly
355 360 365

Glu Leu Thr Ala Glu Gly Leu Arg Thr Arg Leu Glu Arg Val Leu Gly
370 375 380

Glu Pro Ser Phe Gly Thr Ala Ala Gln Ala Leu Ser Asp Thr Ile Ala
385 390 395 400

Ala Glu Pro Ser Pro Ser Glu Val Val Pro Val Leu Glu Glu Leu Thr
405 410 415

Gly Arg His Arg Pro Gly Thr Arg
420

<210> 15
<211> 139
<212> PRT
<213> *Streptomyces nogalater* ATCC 27451

<220>
<223> "translate of *sno*L, function: unknown"

<400> 15

Met Ser Thr Thr Ala Asn Lys Glu Arg Cys Leu Glu Met Val Ala Ala
1 5 10 15

Trp Asn Arg Trp Asp Val Ser Gly Val Val Ala His Trp Ala Pro Asp
20 25 30

Val Val His Tyr Asp Asp Glu Asp Lys Pro Val Ser Ala Glu Glu Val
35 40 45

Val Arg Arg Met Asn Ser Ala Val Glu Ala Phe Pro Asp Leu Arg Leu
50 55 60

Asp Val Arg Ser Ile Val Gly Glu Gly Asp Arg Val Met Leu Arg Ile
65 70 75 80

Thr Cys Ser Ala Thr His Gln Gly Val Phe Met Gly Ile Ala Pro Thr
85 90 95

Gly Arg Lys Val Arg Trp Thr Tyr Leu Glu Glu Leu Arg Phe Ser Glu
100 105 110

Ala Gly Lys Val Val Glu His Trp Asp Val Phe Asn Phe Ser Pro Leu
115 120 125

Phe Arg Asp Leu Gly Val Val Pro Asp Gly Leu
130 135

<210> 16
<211> 155
<212> PRT
<213> *Streptomyces nogalater* ATCC 27451

<220>
<223> "translate of *sno*O, function: homologous to *mtm*X of mithramycin cluster"

<400> 16

Met Ser Val Arg Thr Asp Gln Thr Ala Ala Pro Glu Asp Arg Ala Ala
1 5 10 15

Ala Thr Asp Pro Gly Phe Gly His Leu Tyr Ala Gln Val Gln Gln Phe
20 25 30

Tyr Ala Arg Gln Met Gln Leu Leu Asp Ser Gly Ala Ala Glu Glu Trp
35 40 45

Ala Ala Thr Phe Thr Glu Asp Gly Thr Phe Ala Arg Pro Ser Ser Pro
50 55 60

Glu Pro Ala Arg Gly His Ala Glu Leu Ala Ala Gly Ala Arg Ala Ala
65 70 75 80

Ala Glu Arg Leu Ala Ala Glu Gly Leu Ser His Arg His Val Ile Gly
85 90 95

Met Thr Ala Val Arg Arg Glu Pro Asp Gly Ser Val Phe Val Arg Ser
100 105 110

Tyr Ala Gln Val Phe Ala Thr Arg Arg Gly Glu Ala Pro Arg Leu His
115 120 125

Leu Ile Cys Val Cys Glu Asp Val Leu Val Arg Glu Gly Pro Gly Leu
130 135 140

Lys Val Arg Glu Arg Val Val Thr His Asp Ala
145 150 155

<210> 17
<211> 281
<212> PRT
<213> *Streptomyces nogalater* ATCC 27451

<220>
<223> "translate of *sno*aF, function: C-7 ketoreductase"

<400> 17

Val Arg Ala Met Thr Asp Ser Thr Gly Pro Arg Pro Val Pro Ala Met
1 5 10 15

Ser Pro Ala Pro Ser Pro Thr Pro Ser Pro Gly Pro Ala Pro Gly Ser
20 25 30

Glu Pro Ala Pro Leu Ala Val Ile Val Thr Gly Gly Gly Ser Gly Ile
35 40 45

Gly Arg Ala Thr Ala Arg Ala Phe Ala Ala Gln Gly Ala Lys Val Leu
50 55 60

Val Val Gly Arg Thr Glu Asp Ala Leu Ala Gln Thr Ala Glu Gly Cys
65 70 75 80

Ala Asp Met Arg Val Leu Val Ala Asp Val Ala Ser Pro Asp Gly Pro
85 90 95

Gln Ala Val Val Asn Ala Ala Leu Arg Glu Phe Gly Arg Ile Asp Val
100 105 110

Leu Val Asn Asn Ala Ala Val Ala Gly Met Glu Thr Leu Gln Thr Val
115 120 125

Asp Arg Asp Ala Val Ala Arg Gln Phe Gly Thr Asn Leu Thr Ala Pro
130 135 140

Leu Phe Leu Val Gln Ser Ala Leu Gly Ala Leu Glu Lys Ser Arg Gly
145 150 155 160

Ile Val Val Asn Val Gly Thr Ala Ala Thr Leu Gly Leu Arg Ala Ala
165 170 175

Pro Thr Gly Ala Leu Tyr Gly Ala Ser Lys Val Ala Leu Asp Tyr Leu
180 185 190

Thr Arg Thr Trp Ala Val Glu Leu Ala Pro Arg Gly Ile Arg Val Val
195 200 205

Gly Val Ala Pro Gly Val Ile Asp Thr Gly Ile Gly Val Arg Met Gly
210 215 220

Met Thr Pro Glu Gly Tyr Arg Glu Phe Leu Thr Gly Met Gly Gly Arg
225 230 235 240

Val Pro Val Gly Arg Val Gly Arg Pro Glu Asp Val Ala Trp Trp Ile
245 250 255

Val Gln Leu Ala Arg Pro Glu Ala Gly Tyr Ala Thr Gly Met Val Val
260 265 270

Pro Val Asp Gly Gly Leu Ser Leu Val
275 280

<210> 18
<211> 190
<212> PRT
<213> *Streptomyces nogalater* ATCC 27451

<220>
<223> "translate of *sno*N, function: unknown"

<400> 18

Val Gln Glu Thr Glu Pro Gly Val Pro Ala Asp Leu Pro Ala Glu Ser
1 5 10 15

Asp Pro Ala Ala Leu Glu Arg Leu Ala Ala Arg Tyr Arg Arg Asp Gly
20 25 30

Tyr Val His Val Pro Gly Val Leu Asp Ala Gly Glu Val Ala Glu Tyr
35 40 45

Leu Ala Glu Ala Arg Arg Leu Leu Ala His Glu Glu Ser Val Arg Trp
50 55 60

Gly Ser Gly Ala Gly Thr Val Met Asp Tyr Val Ala Asp Ala Gln Leu
65 70 75 80

Gly Ser Asp Thr Met Arg Arg Leu Ala Thr His Pro Arg Ile Ala Ala
85 90 95

Leu Ala Glu Tyr Leu Ala Gly Ser Pro Leu Arg Leu Phe Lys Leu Glu
100 105 110

Val Leu Leu Lys Glu Asn Lys Glu Lys Asp Ala Ser Val Pro Thr Ala
115 120 125

Pro His His Asp Ala Phe Ala Phe Pro Phe Ser Thr Ala Gly Thr Ala
130 135 140

Leu Thr Ala Trp Val Ala Leu Val Asp Val Pro Val Glu Arg Gly Cys
145 150 155 160

Met Thr Phe Val Pro Gly Ser His Leu Leu Pro Asp Pro Asp Thr Gly
165 170 175

Asp Glu Pro Trp Ala Gly Ala Phe Thr Arg Pro Gly Glu Ile
180 185 190